17 September 2020
EMA/CHMP/522273/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Orfadin

International non-proprietary name: nitisinone

Procedure No. EMEA/H/C/000555/II/0071

Note

Variation assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

ADR: adverse drug reaction

AE: Adverse Events

AKU: alkaptonuria

AKUSSI: Alkaptonuria Severity Score Index

ALP: alkaline phosphatase

ATC: Anatomical, Therapeutical, Chemical

BP: Blood Pressure

BQA: benzoquinone acetic acid

Cav: concentration average

CHMP: Committee for Medicinal Products for Human Use

CI: Confidential Interval

Cmax: concentration maximum

CRF: Case Report Form

CSP: clinical study protocol

DEXA: Dual energy X-ray absorptiometry

eGFR: estimated glomerular filtration rate

FAH: Fumarylacetoacetate hydrolase

FAS: Final Analysis Set

F_{PEN}: Penetration Factor

GCP: Good Clinical Practice

HAQ: Health Assessment Questionnaire

HGA : homogentisic acid

HGD: homogentisate 1,2-dioxygenase

HPP: 4-Hydroxyphenylpyruvate

HPPD: 4-Hydroxyphenylpyruvate dioxygenase

HT-1: hereditary tyrosinemia type 1

LLOQ: Lower Limit of Quantitation

Log Kow: Octanol/Water partitioning coefficient

LS: Least Square

MAR : missing at random

MCMC: Markov-Chain Monte Carlo

MMRM: Mixed effect model repeat measurement

MNAR : missing not at random

MRI: Magnetic Resonance Imaging

PBT: Persistent, Bioaccumulative and Toxic

PECsw: Predicted Environmental Concentration surface water

p-HGA : plasma concentration of HGA

PT: Preferred Term

QoL: Quality of Life

REML: Restricted maximum likelihood method

SA: Scientific Advice

SAE: Serious Adverse Event

SAP: Statistical Analysis Plan

SE: standard error

SF36: Short Form Health Survey

SmPC: Summary of Product Characteristics

SOC: Standard Organ Class (SOC)

u-HGA: urine concentration of HGA

u-HGA24: urine concentration of HGA in 24h.

ULN: Upper Limit of Normal

WHO: World Health Organization

WOMAC: Wester Ontario and McMaster Universities Arthritis

1. Background information on the procedure

1.1. Type II variation

Pursuant to Article 16 of Commission Regulation (EC) No 1234/2008, Swedish Orphan Biovitrum International AB submitted to the European Medicines Agency on 10 February 2020 an application for a variation.

The following variation was requested:

Variation requested		**Type**	**Annexes affected**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	Type II	I and IIIB

Extension of indication to include treatment of adult patients with alkaptonuria (AKU) for Orfadin; as a consequence, sections 4.1, 4.2, 4.4, 4.6, 4.8, 5.1 and 10 of the SmPC are updated. The Package Leaflet is updated in accordance. Version 5.2 of the RMP has also been submitted accordingly and includes an update in accordance with GVP Module V Revision 2.

The variation requested amendments to the Summary of Product Characteristics and Package Leaflet and to the Risk Management Plan (RMP).

Information on paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the MAH did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

MAH request for additional data exclusivity

The MAH requested consideration of its application in accordance with Article 10(5) of Directive 2001/83/EC - one year of data exclusivity for a new indication.

Scientific advice

The MAH seeks Scientific advice at the CHMP.

1.2. Steps taken for the assessment of the product

The Rapporteur appointed by the CHMP was:

Rapporteur: Daniela Melchiorri

Timetable	Actual dates
Submission date	10 February 2020
Start of procedure:	29 February 2020
CHMP Rapporteur Assessment Report	24 April 2020
Updated PRAC Rapporteur Assessment Report	6 May 2020
PRAC members comments	6 May 2020*
PRAC Outcome	14 May 2020
CHMP members comments	18 May 2020*
Updated CHMP Rapporteur(s) (Joint) Assessment Report	20 May 2020
Request for supplementary information (RSI)	28 May 2020
CHMP Rapporteur Assessment Report	17 August 2020
PRAC Rapporteur Assessment Report	17 August 2020
PRAC members comments	26 August 2020*
Updated PRAC Rapporteur Assessment Report	27 August 2020
PRAC Outcome	3 September 2020
CHMP members comments	7 September 2020*
Updated CHMP Rapporteur Assessment Report	10 September 2020
CHMP opinion	17 September 2020
The CHMP adopted a report on the novelty of the indication in comparison with existing therapies and the significant non-clinical or clinical data in relation to the claimed new indication for Orfadin (Appendix x)	17 September 2020

* planned dates

2. Scientific discussion

2.1. Introduction

2.1.1. Problem statement

Disease or condition

AKU is a serious, autosomal recessive, multisystem disorder affecting approximately one in every 250 000 to 1 million people. Of 626 patients identified worldwide, there are 358 patients in Europe, 208 of whom are found in Slovakia. Morbidity in alkaptonuria (AKU) is caused by increased levels of the tyrosine metabolite homogentisic acid (HGA) due to a deficient enzyme, homogentisate 1,2-dioxygenase (HGD), that is the third enzyme involved in tyrosine catabolism.

The absence of HGD results in patients being unable to fully metabolize the amino acid tyrosine, leading to high serum levels of HGA. Despite efficient and marked urinary excretion of much of the HGA, some of it is oxidized to a melanin-like pigment (via BQA). This pigment is deposited in connective tissues, particularly cartilage, a process termed ochronosis, with darkening of cartilaginous tissues and bone, arthritis and joint destruction, and deterioration of cardiac valves. There are few clinical features, aside from dark urine, until the late 20s or early 30s when progressive arthritic pain, affecting the spine and synovial joints, large and small, as well as pigmentation of eyes (sclera) and ear cartilage, begins. Fifty percent (50%) of patients require at least one joint replacement by age 55 years [Phornphutkul et al 2002].

Currently, there is no pharmacological treatment approved available for patients with AKU and treatment options are limited to treatment of the disease sequelae as they arise, including physiotherapy, surgery and analgesia.

AKU is a very rare disease without any approved drug. While it is true that life expectancy does not seem to be reduced, the disease is characterized by multiple manifestations, particularly related to joints, with development of joint pain, movement limitations, and half patients in need of at least one joint replacement by 55 years. Therefore, effective therapy for AKU is considered by the CHMP as an unmet medical need.

The sought indication is: "Orfadin is indicated for the treatment of adult patients with alkaptonuria (AKU)".

2.1.2. About the product

Nitisinone is a competitive inhibitor of the enzyme, 4-Hydroxyphenylpyruvate dioxygenase (HPPD), which metabolizes 4-Hydroxyphenylpyruvate (HPP) to HGA. It has been shown to reduce urinary excretion and serum levels of HGA in a dose- and concentration-dependent manner in patients with AKU. It is hypothesized that if HGA levels are reduced to, and maintained at, normal, or near normal levels in AKU patients before the onset of overt ochronosis (i.e., blue-black pigmentation), this might prevent the development of the debilitating clinical features of the disease.

Likewise, it is hypothesized that treatment with nitisinone in patients who have already developed some degree of ochronosis, would slow down further progression of ochronosis and thereby reduce the incidence of disease related consequences.

Nitisinone (Orfadin) is currently approved for the treatment of hereditary tyrosinemia type 1 (HT-1), where it acts by the same mechanism, i.e., by inhibition of HPPD which prevents formation of toxic metabolites further down in the tyrosine metabolic pathway. Due to the inhibition of HPPD, treatment with nitisinone leads to increased serum concentrations of tyrosine. High levels of tyrosine may lead to ocular signs and symptoms and adverse effects on the skin.

2.1.3. The development programme/compliance with CHMP guidance/scientific advice

The main issues discussed during the SA procedure were:

i. **The acceptability of a clinical development program based mainly on a pharmacodynamic variable: the normalization of plasma/urinary levels of HGA.** The CHMP advised that, although only HGA is the cause of the ochronosis process that in turn results in the co-morbidities effects of alkaptonuria, there is no historical clinical data to support the assumption that the control of HGA levels in patients with AKU will arrest ochronosis. Therefore, while a primary endpoint based on a pharmacodynamic variable may be accepted, to support the claimed indication, data on treatment effect on clinical outcomes, and on safety will need to be provided at the time of MAA. It is expected that at the time of submission, the Applicant will be able to build a persuasive direct link from HGA levels through ochronosis to clinical outcome. The characterization of the natural course of disease, as well as a detailed justification on the normal HGA levels will be key basis for an approval in AKU patients based on the HGA surrogate biomarker instead of the expected robust clinical data in standard conditions

ii. **The initially proposed cut-off point of 3.0 µM (0.5 µg/ml) for plasma-HGA**. The CHMP recommended to use physiological levels of HGA as an endpoint, and to switch to measurements in urine, since plasma levels are much below the lower limit of quantitation in standard bioanalytical methods and, also, urine data are less variable. Two papers with data on normal levels of u-HGA are currently available: 20 - 30 mg/day (Introne et al, 2011) and < 100 mg/day (Suwannarat et al. 2005). The published literature being inconsistent, there is the need to investigate normal u-HGA levels. This is as important information in the authorisation process.

iii. **The acceptability of the All Alkaptonuria Severity Score Index (AKUSSI) to measure treatment effect on AKU disease.** This scale measures AKU disease severity in clinical, joint and spine domains. Although validated, this scoring system bears some limitations in its use in clinical trials, and the CHMP recommended to implement further measures to confirm treatment benefit on clinical outcomes. During the Discussion Meeting the Applicant presented a more comprehensive plan to assess clinical effect after 12 and 48months of treatment including the following variables: modified cAKUSSI scores (i.e. without measures of pigmentation: mAKUSSI), pain (VAS), total cAKUSSI, all individual cAKUSSI items, quality of life (SF36),health assessment (HAQ), pain, stiffness, and physical function (WOMAC index), range of hip motion, and aortic valve gradients. The updated plan was considered overall acceptable by the CHMP. In particular, the use of mAKUSSI over AKUSSI was advised.

Overall, the development program of the product was in compliance with the CHMP Scientific Advice.

2.1.4. General comments on compliance with GCP

As claimed by the applicant all the studies were conducted in accordance to GCP.

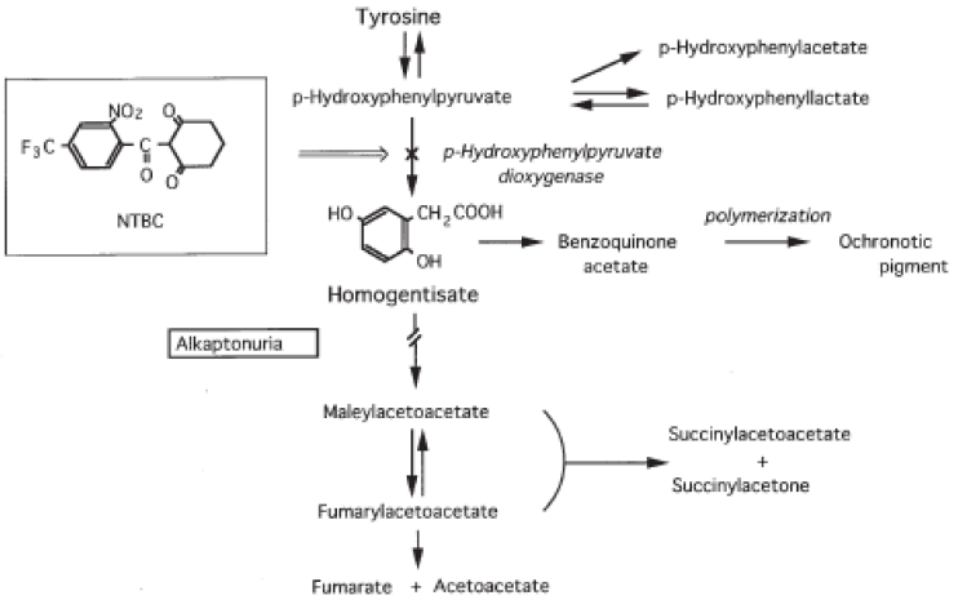
2.2. Non-clinical aspects

2.2.1. Introduction

Published nonclinical pharmacodynamic data that demonstrate that nitisinone has compelling therapeutic effects on the development of ochronosis in animal models of AKU. These are summarized below.

2.2.2. Pharmacology

AKU is an inborn error of metabolism, caused by a deficiency of HGD, an enzyme involved in the breakdown of HGA, and an intermediate of the metabolism of tyrosine and phenylalanine (see Figure 1). It arises in humans from homozygous or compound heterozygous mutations in the HGD gene (Fernandez-Canon et al., 1996). At least 129 different human mutations have been identified (Nemethova et al., 2016). The loss of enzymatic activity leads to an increase and accumulation of the systemic content of HGA, and the renal excretion of HGA increases dramatically. The treatment with nitisinone is suggested to abolish this systemic increase in HGA, and thereby to prevent the development of the debilitating ochronosis of joints, bone and other tissues (La Du et al., 1958).



NTBC, also known as nitisinone.
From Suzuki et al 1999 (9).

Figure 1. Metabolic pathway of tyrosine

Primary pharmacodynamic studies

In vitro data

The role of HGA in causing ochronosis has been demonstrated in a series of in vitro studies. Cell culture models, both of chondrocytes isolated from the joints of patients with AKU and of osteosarcoma cell lines, show that elevated HGA concentrations are the prime driver of ochronosis (Tinti et al., 2011; Braconi et al., 2012;). Osteosarcoma cell cultures in the presence of HGA accumulate ochronotic pigment in a dose-dependent fashion, whilst the pigment is absent in cells cultured in the absence of HGA (Tinti et al., 2011). Cell cultures of primary chondrocytes isolated from both fetal and adult rabbit cartilage demonstrate that incubation with HGA causes dose-dependent cytotoxicity with concomitant compromise of the cultures' ability to synthesize proteoglycans (Kirkpatrick et al., 1994).

Nitisinone has been demonstrated, both in vitro and in vivo, to be a potent inhibitor of hepatic HPPD in rodents, thus preventing the formation of downstream metabolites, including HGA (Ellis et al., 1995).

Treatment with nitisinone is therefore likely to prevent ochronosis and subsequent clinical symptoms of AKU.

In vivo data

An AKU mouse model was developed in 1994 (Montagutelli et al., 1994). It was created by ethylnitrosourea-induced mutagenesis. Homozygous (HGD-/-) mice were demonstrated to have high levels of HGA in the urine and almost no HGD activity in the liver, but no clinical signs of ochronosis were present.

Therapeutic effects of nitisinone were demonstrated for the first time using this AKU mouse model (Suzuki et al., 1999). The urinary output of HGA was reduced to less than 2 % of the pre-treatment level 12 to 24 hours after a single oral dose by stomach tube of 100 µg nitisinone, i.e., approximately 5 mg/kg bodyweight. After repeated daily dosing of 25 µg nitisinone orally for 4 weeks, i.e., approximately 1.25 mg/kg bodyweight, the reduction in urinary levels of HGA was preserved throughout the treatment period. A 3-fold increase in the plasma levels of tyrosine was recorded in the nitisinone-treated normal animals when compared to the pre-treatment levels, and by 4 to 5 times in nitisinone-treated AKU mice. X-ray examination of 3 AKU mice at age 13 months showed no calcification of the cartilage and no osteoarthritic changes in the knee and spine.

Despite initial difficulties in demonstrating ochronosis in the above mouse model (HGD-/-), a later study verified that these mice did indeed develop ochronosis (Preston et al., 2014). Samples were taken for p-HGA measurements at 2- or 4-weekly intervals between ages 5 and 79 weeks. Wild type p-HGA levels were below the limit of detection for the analytical method. The mean p-HGA concentration in AKU mice was elevated; the mean (SE) lifetime level was 0.148 (0.019) mmol/L (range, 0.768 to 0.023 mmol/L), with no obvious changes with age.

The authors were able to histologically show the presence of ochronosis in tissue specimens taken from the AKU mice by using a modified Schmorl's stain. Extensive chondrocytic pigmentation of the femoral and tibial calcified cartilage was observed.

A cohort of the AKU mice were exposed to nitisinone via the drinking water ad libitum from 8 to 67 weeks of age. No plasma samples for drug level measurements were taken to confirm exposure to nitisinone. The treated mice showed a significant reduction in plasma HGA between 8 and 14 weeks of age when compared with untreated controls ($p < 0.001$). By using the modified Schmorl's stain, the joint ochronosis could be quantified and the treatment with nitisinone was demonstrated to completely prevent the chondron pigmentation.

Another study (Keenan et al., 2015) showed that administration of nitisinone to the AKU mice arrests further deposition of ochronotic pigment in the tibiofemoral joint but did not result in the clearance of existing pigment.

The original AKU mouse model (Montagutelli et al., 1994) was further improved by knocking out two genes in the mouse; the gene of tyrosinemia type 1 (HT-1) and the HGD gene (Manning et al., 1999). The resultant HGD+/-/FAH-/- animal could be maintained in a healthy state if treated with nitisinone.

Withdrawal of nitisinone was most often lethal, but in some cases the animal survived due to production of homozygosity of the HGD mutation in a process called reversion. The animals developed ochronosis of the kidney, the distal femur and proximal fibula, as evidenced by the presence of chondrocytes-bearing ochronotic pigment (Taylor et al., 2012).

2.2.3. Ecotoxicity/environmental risk assessment

The Environmental Risk Assessment of nitisinone, conducted, as stated by the MAH in accordance with Guideline on the environmental risk assessment of medicinal products of human use EMEA/CHMP/SWP/4447/00 Rev. 1, resulted in PECsw < 0.01 µg/L when using a refined FPEN based on the highest regional prevalence for the indications HT-1 and AKU. In addition, the PBT screening shows a log Kow below the limit of 4.5. Results led to the MAH conclusion, that the medicinal product is unlikely to represent a risk for the environment following its prescribed usage in patients. However, the logKow determination was not considered acceptable by the CHMP since the submitted study was not in line with the requirements of OECD 107.

2.2.4. Discussion on non-clinical aspects

Nitisinone pharmacological effect is supported by bibliographical non clinical data. AKU disease mouse model (HGD-/-) were demonstrated to develop ochronosis. Treatment with nitisinone lowered the HGA levels in blood and urine, decreasing therefore the rate of formation of ochronotic pigment in the tibiofemoral joint. Similar results were obtained with the HGD+/-/FAH-/- animal model, in which nitisinone administration was able to revert the incipient ochronosis associated with single chondrocytes and the immediate pericellular matrix, which mirrors authentically the early stages of the process in humans and mirrors closely that seen in the HGD -/- animals described above. Overall, data adequately showed that this mouse model may accurately depict the HGA-induced ochronosis seen in the human pathology of AKU and further show the preventive effects of nitisinone on the ochronotic process. The ERA is not complete and the CHMP recommended the MAH to provide further data to substantiate that the present extension of indication for the medicinal product is unlikely to represent a risk for the environment.

2.2.5. Conclusion on the non-clinical aspects

The ERA data available at the initial marketing authorisation was updated in this application. However, it is not complete to conclude on whether a significant increase in environmental exposure is expected further to the extended use of nitisinone.

In the context of the obligation of the MAH to take due account of technical and scientific progress, the CHMP recommends the following points are recommended for further investigation:

In order to complete the Phase I ERA, an experimentally determined log Kow of the active substance Nitisinone should be provided in accordance with the Q&A document by the European Medicines

Agency (EMA) Questions and answers on 'Guideline on the environmental risk assessment of medicinal products for human use' (EMA/CHMP/SWP/44609/2010).

2.3. Clinical aspects

The pharmacokinetic profile and pharmacological activity of Orfadin has been well characterised in humans. New pharmacokinetic and pharmacodynamic data in the AKU population have been collected through the dose-response and main pivotal studies, SONIA-1 and SONIA-2, which is considered acceptable by the CHMP.

2.3.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the MAH.

- Tabular overview of clinical studies

Study ID	No. of study centres / locations	Design	Study Posology	Study Objective	Subjs by arm entered/ compl.	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
SONIA-1	Multicenter 3 EU sites	Randomized, open-label, no-treatment controlled parallel	1, 2, 4, 8 mg	To identify the nitisinone dose that lowers urinary excretion of HGA to normal, or near normal, levels.	Nitisinone 32; Control 8	4 weeks		AKU Age ⩾18 years	u-HGA24
SONIA-2	Multicenter 3 EU sites	Randomized, open-label, no-treatment controlled parallel	10 mg	To demonstrate an effect of nitisinone on HGA formation and clinical outcome parameters, and long-term safety.	Nitisinone 69; Control 69	4 years		AKU with clinical manifestations Age ⩾25 years	

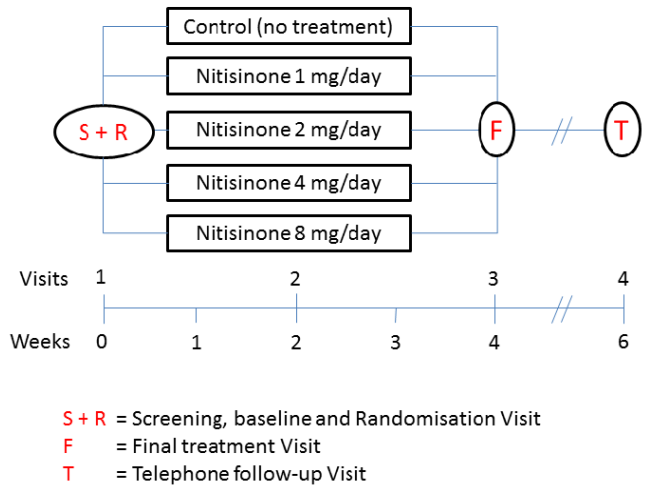
u-HGA_{24} = urinary HGA in 24h.

2.4. Clinical efficacy

2.4.1. Dose response study

The dose response study, **SONIA-1**, investigating the effect of different doses of once daily nitisinone on 24-hour urinary homogentisic acid excretion (u-HGA24) in patients with alkaptonuria after 4 weeks of treatment. The intention of the study was to identify the nitisinone dose that lowers urinary excretion of HGA to normal, or near normal, levels. A separate study in non-AKU subjects was performed in parallel in order to define normal levels of both urinary excretion and serum levels of HGA.

Patients were randomized to receive either 1 mg, 2 mg, 4 mg or 8 mg nitisinone once daily or no treatment (control). 40 patients were planned to be randomized, equally distributed, to the groups (8 patients/group). The overall design of the dose-response study is presented in the Figure 2.



S + R = Screening, baseline and Randomisation Visit
F = Final treatment Visit
T = Telephone follow-up Visit

Source: CSR SONIA 1, Section 6.1, Figure 1.

Figure 2. Overall design of SONIA-1 study

The primary endpoint was u-HGA24 after 4 weeks of treatment with nitisinone. Secondary endpoints included 24-hour serum concentration of HGA (s-HGA) and serum concentration of Tyrosine (s-Tyr) profiles at Week 4 and nitisinone steady state pharmacokinetic variables.

Results for u-HGA24 are shown below in Table 1, Figures 3 and 4

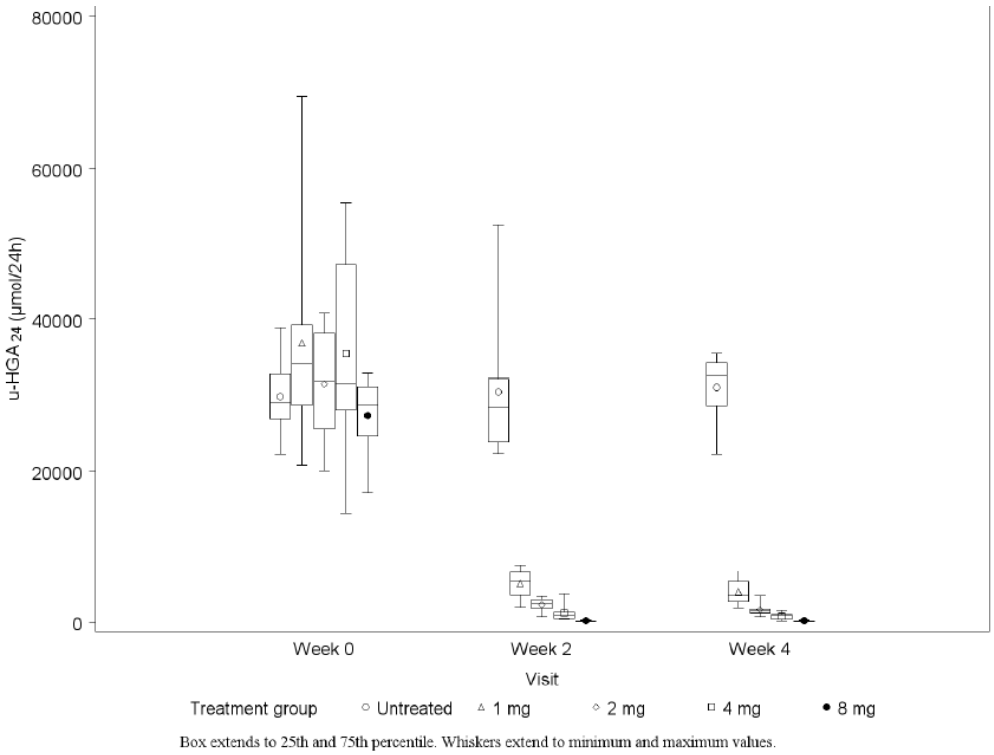
Table 1. u-HGA24. MMRM (FAS)

Visit	Statistic	Untreated (N=8)	1 mg (N=8)	2 mg (N=8)	4 mg (N=8)	8 mg (N=8)
Week 0	n	8	8	8	8	8
	Mean	29835.8	36757.0	31439.8	35453.4	27353.5
	SD	5067.0	14614.4	7388.0	13580.9	5234.9
	Median	29041.3	34034.4	31754.7	31502.7	28728.7
	min	22270	20743	19994	14443	17075
	max	38832	69503	40747	55394	32897
Week 2	LS Mean	30563.1	4968.1	2341.4	1107.4	315.9
	SE	2718.8	377.6	368.6	373.8	373.9
	95 % CI, lower	24630.5	4207.9	1599.6	355.1	-436.7
	95 % CI, upper	36495.6	5728.2	3083.2	1859.7	1068.5
Week 4	LS Mean	31152.2	3846.1	1667.6	685.6	326.7
	SE	2718.8	377.6	368.6	373.8	373.9
	95 % CI, lower	25219.7	3085.9	925.8	-66.7	-425.8
	95 % CI, upper	37084.8	4606.2	2409.3	1437.9	1079.3

LS Mean: Least square mean
Source: Table 10.2 - 2

FAS: Full analysis set, MMRM: Mixed effect model repeat measurement

HGA excretion decreased in a clear dose-dependent manner, and at Week 4 the least square (LS) mean values were reduced by approximately 90, 95, 98 and 99% compared to the values at baseline in patients receiving nitisinone 1, 2, 4 or 8 mg, respectively. No change in u-HGA24 was seen in the untreated control group.

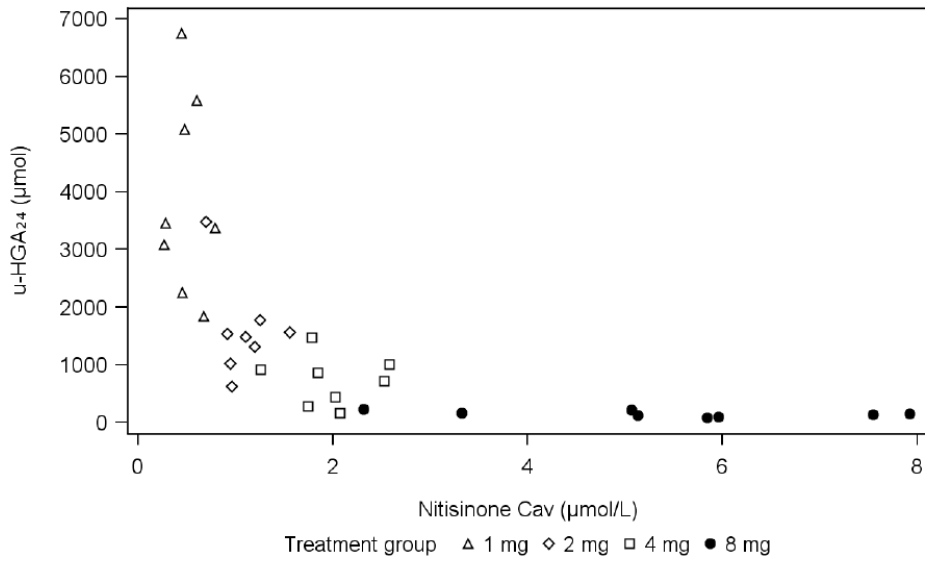


Source: Figure 10.2 - 3

Figure 3. u-HGA24 over time (FAS)

Urinary HGA was only quantifiable in 7 of the 22 individuals in the separate study in non-AKU subjects. The highest 24-hour excretion observed in any of those 7 subjects was 2.91 µmol. Thus, no patient in the present study achieved a u-HGA24 level as low as the highest observed value in any normal subject. The LS mean excretion at Week 4 in the 8-mg group (=327 µmol) is about 100 times higher than that value, despite the 99% reduction of u-HGA24 from baseline in that dose group.

The relationship between u-HGA24 and average concentration of serum nitisinone is shown in the figure 4. A clear concentration-response relationship can be seen for u-HGA up to an average nitisinone concentration of about 3 µmol/L. For C_{av} values above 3 µmol/L there was apparently no further decrease in u-HGA.



Source: Figure 10.2 - 5

Figure 4. Urine HGA in relation to average concentration (Cav) of serum nitisinone

Results for serum HGA and Tyrosine profiles at Week 4 are presented in Tables 2 and 3. The relationship between s-HGA and average concentration for nitisinone could not be determined due to several s-HGA values below the LLOQ. The relationship between C_{av} for s-Tyr and C_{av} for nitisinone at Week 4 is shown below in Figure 5.

Table 2. Average and maximum serum concentrations of HGA at Week 4 (FAS)

	Statistic	Untreated (N=8)	1 mg (N=8)	2 mg (N=8)	4 mg (N=8)	8 mg (N=8)
C_{av} (µmol/L)	n	8	8	8	8	8
	Mean	35.34	4.11	ND	ND	ND
	SD	12.12	1.93	ND	ND	ND
	Median	36.72	3.93	3.32	<3.1	<3.1
	min	21.0	0.5	<3.1	0.1	<3.1
	max	52.0	7.3	5.8	1.3	0.4
C_{max} (µmol/L)	n	8	8	8	8	8
	Mean	58.74	7.11	ND	ND	ND
	SD	17.39	2.13	ND	ND	ND
	Median	53.40	6.60	5.10	3.40	<3.1
	min	34.6	4.3	<3.1	<3.1	<3.1
	max	89.5	10.7	7.5	4.7	3.2

Source: Table 10.2 - 8

Several descriptive statistics, such as mean and standard deviation, could not be calculated for the 2-, 4- and 8-mg dose groups due to a total of 14 individuals with values below the Lower Limit of Quantification (LLOQ). Despite this, an indication of a dose-response relationship is seen for the C_{max} values. A dose-response relationship is also reflected by the increasing number of patients with values below the LLOQ with increasing doses. There were 3 patients on 2 mg, 4 patients on 4 mg and 7 patients on 8 mg without a single quantifiable concentration in the samples collected over the 24-hour

dosage interval at Week 4. Serum levels of HGA were non-quantifiable in all non-AKU subjects. It was not possible to determine if any of the nitisinone-treated AKU patients reached normal levels of s-HGA.

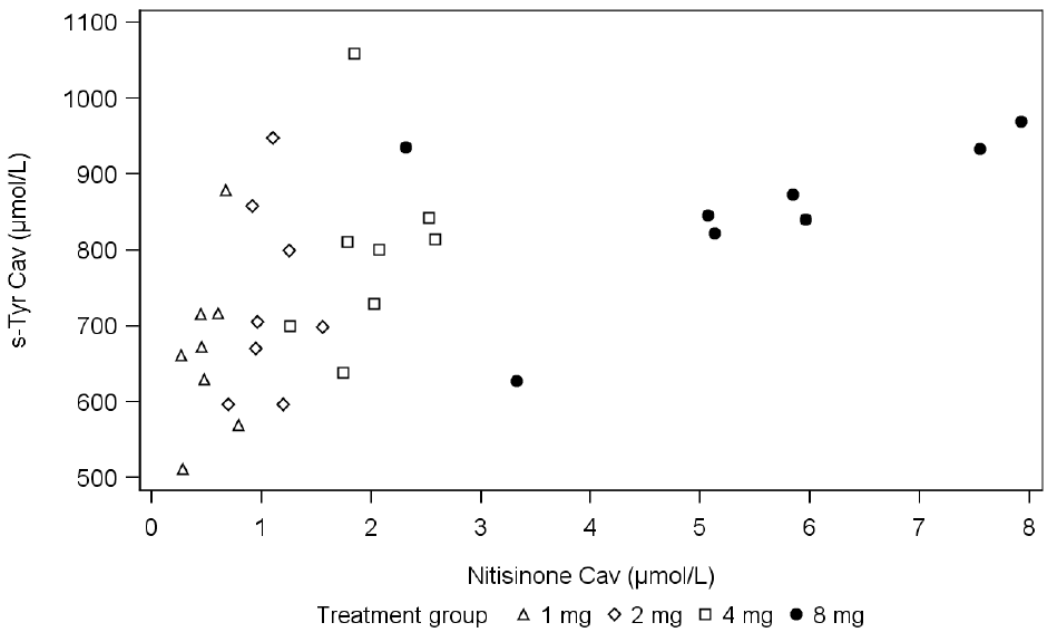
Table 3. Average and maximum serum concentrations of tyrosine at Week 4 (FAS)

Table 10 Average and maximum serum concentrations of tyrosine at Week 4 (FAS)

	Statistic	Untreated (N=8)	1 mg (N=8)	2 mg (N=8)	4 mg (N=8)	8 mg (N=8)
C_{av} (µmol/L)	n	8	8	8	8	8
	Mean	60.7	669.5	734.2	799.6	856.1
	SD	9.8	110.1	125.2	125.5	106.5
	Median	58.0	667.3	702.1	805.9	860.0
	min	52	511	596	639	627
	max	83	879	948	1059	970
C_{max} (µmol/L)	n	8	8	8	8	8
	Mean	75.8	737.9	828.1	878.8	941.9
	SD	8.2	114.5	126.2	137.3	106.8
	Median	74.0	721.0	835.5	889.0	984.0
	min	68	594	662	696	715
	max	92	978	1010	1155	1066

Source: Table 10.2 - 11

s-Tyr levels increased with nitisinone dose, but the dose-response relationship appear less pronounced than for uHGA24 vs dose. At all doses, nitisinone increased s-Tyr levels above 500 µmol/L, which is the recommended threshold specified in the SmPC of Orfadin in the HT-1 indication.



Source: Figure 10.2 - 13

Figure 5. Cav for s- Tyrosine vs Cav for nitisinone at Week 4 (FAS)

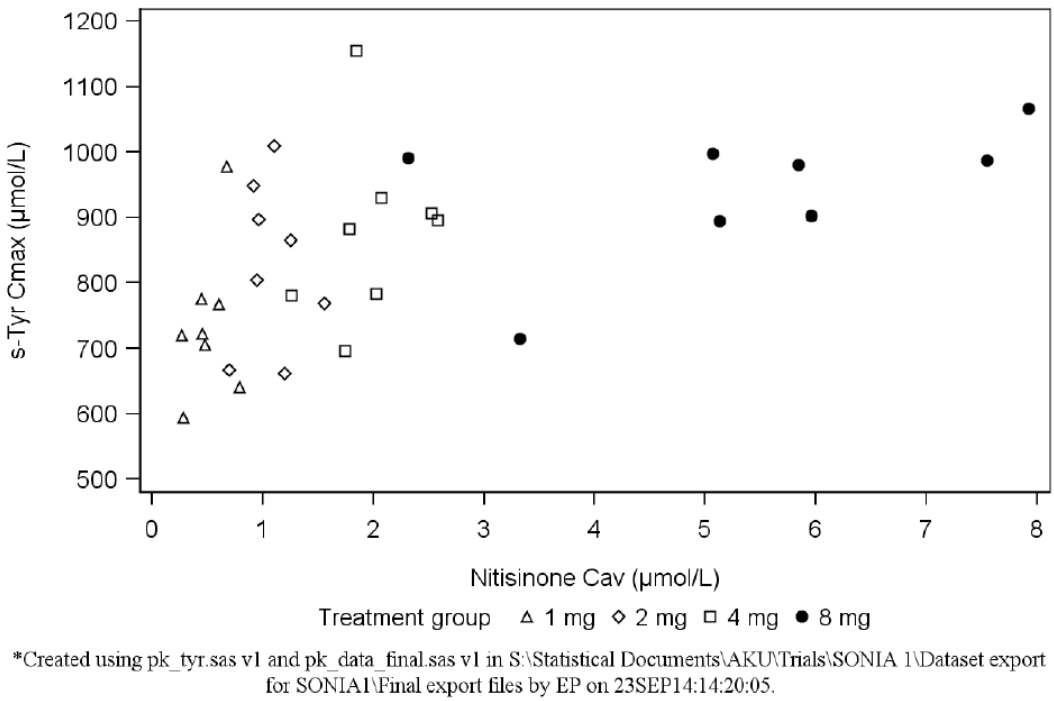


Figure 6. s- Tyrosine Cmax vs C_{av} for nitisinone Cav at Week 4 (FAS)

All doses, including the 1-mg dose, resulted in daily average levels above 500 µmol/L in all individuals. From a nitisinone C_{av} of about 5 µmol/L and upward, all average s-Tyr were at least 800 µmol/L (Figure 6).

2.4.2. Main study

The main study, **SONIA 2**, is an international, multicenter, randomized, evaluator-blinded, no-treatment controlled, parallel-group study to assess the efficacy and safety of once daily nitisinone in patients with alkaptonuria after 12 months of treatment, followed by an additional 36-month treatment period.

Methods

Study participants

Study centers were as follows: Royal Liverpool University Hospital, Liverpool, UK. National Institute of Rheumatic Diseases, Piešťany, Slovakia Hôpital Necker-Enfants Malades, Paris.Cedex, France.

Main inclusion criteria were as follows: confirmed diagnosis of AKU based on elevated uHGA level; any clinical manifestations of AKU, such as clinical ochronosis or chronic back / joint pain and age ≥ 25 years. Exclusion criteria included: treatment with nitisinone within 3 months of randomization, or participation in another clinical study within 3 months of randomization; female patient of child-bearing potential not using a reliable method of contraception, or currently pregnant or lactating; current malignancy; unstable cardiovascular disease; serum potassium < 3.0 mmol/L, or eGFR < 60 mL/min,

or ALT > 3 x ULN, or Hemoglobin < 10.0 g/dL, or Platelets < 100 x 109/L, or Total white blood count < 3.0 x 10^9/L or neutrophil count < 1.5 x 10^9/L.

A patient was to be withdrawn from treatment if the patient developed ocular signs or symptoms, or a skin rash (hyperkeratotic lesions). These AEs were judged by the investigator to be related to elevated tyrosine. Patients withdrawn due to an AE could not re-enter the study. Patients temporarily withdrawn from treatment due to suspected tyrosine toxicity could, however, continue in the study on a lower dose of nitisinone (2 mg) at the discretion of the investigator once all signs and symptoms of tyrosine toxicity had resolved. Patients with temporarily withdrawn treatment due to a suspected, but not later confirmed, tyrosine toxicity, could continue in the study on the 10-mg dose. In both cases, dates for temporary withdrawal of nitisinone, the date when treatment was re-initiated, and the dose used after the pause, were recorded in the CRF. Withdrawn patients were not replaced.

Female patients were to use a reliable method of contraception during the study and for one month after the study. All patients were advised to limit their protein intake in order to keep tyrosine levels as low as possible without introducing specific diets. The importance of controlling protein and phenylalanine intake was emphasized and a list of foods particularly high in tyrosine and phenylalanine was provided. Patients were allowed to continue on any chronic medication but were not to make any changes in the dosage from one month before entering the study and until after the follow-up visit, unless necessary for medical reasons.

Treatments

The dose of nitisinone used in this study was 10 mg/day and was determined after evaluation of the SONIA 1 study results.

In case a patient developed tyrosine-related adverse events, nitisinone dose could be decreased to 2 mg/day.

Nitisinone was taken once daily, in the morning.

Objective

The primary objective was to demonstrate that nitisinone is superior compared to no treatment in reducing u-HGA24 in patients with AKU after 12 months.

Outcomes/endpoints

The primary endpoint was u-HGA24 after 12 months.

Secondary endpoints included: u-HGA24 after 3, 24, 36 and 48 months; occurrence of achieved target level (<300 µmol) of u-HGA24 at 3, 12, 24, 36 and 48 months; predose s-HGA at 3, 12, 24, 36 and 48 months; change from baseline in cAKUSSI, mAKUSSI, scores at 12, 24, 36 and 48 months; change from baseline of joint and spine motion, other pre-defined rheumatology assessments at 12, 24, 36 and 48 months; change from baseline in QOL measured by SF-36 at 12, 24, 36 and 48 months; pre-dose serum nitisinone at 3, 12, 24, 36 and 48 months.

The composite efficacy variable AKUSSI incorporates multiple, clinical AKU outcomes that can be described in a single score, ie eye and ear pigmentation, kidney and prostate stones, aortic stenosis,

bone fractures, tendon/ligament/muscle ruptures, kyphosis, scoliosis, and joint replacements and other manifestations of AKU.

Two types of AKUSSI score were used as secondary outcomes in SONIA 2; the cAKUSSI and a modified version, mAKUSSI, which does not include the pigmentation parameters. The mAKUSSI was agreed with the CHMP during the Scientific Advice. The AKUSSI scoring system is shown in the Table 4 below. For each item, the actual results of measurements were reported in the CRF and used in the statistical analysis for the individual item and in the calculation of the AKUSSI score. All items included in the AKUSSI were assessed at baseline and yearly thereafter.

Table 4. Clinical AKU Severity Score Index (cAKUSSI)

Feature		Score	Feature		Score
CLINICAL FEATURES NON-SPINE NON-RHEUMATOLOGIC					
Eye pigment					
Right eye (Nasal)	Slight	4	**Left eye (Nasal)**	Slight	4
	Marked	8		Marked	8
Right eye (Temporal)	Slight	4	**Left eye (Temporal)**	Slight	4
	Marked	8		Marked	8
Ear pigment					
Right ear	Slight	2	**Left ear**	Slight	2
	Marked	4		Marked	4
Stones					
Prostate Stones	Per episode	4	**Renal Stones**	Per episode	4

Feature		Score	Feature		Score
Musculoskeletal					
Osteopenia hip (bone density)	Grade (T-scores) -1.0 to -1.7 -1.8 to -2.4 ≤ -2.5	 2 4 6			
Adult fracture	Per fracture	8	**Ligament rupture**	Per rupture	8
Tendon rupture	Per rupture	8	**Muscle rupture**	Per rupture	8
Heart					
Aortic sclerosis		4	**Aortic stenosis**	Mild Moderate Severe	8 10 12
ENT					
Hearing impairment	Grade on audiometry (dB loss), per ear 21-35 (mild) 36-60 (moderate) >60 (severe)	 1 2 4	**Dark eardrum**	Per ear	6

NON-SPINE RHEUMATOLOGY					
Clinical joint pain (1 for each large joint area; hips, knees, ankles, feet, shoulders, elbows, wrists & hands - right and left sides = 14 joint areas)					Max 14
Non-spine osteoarticular disease (2 for each large joint area; hips, knees, ankles, feet, shoulders, elbows, wrists & hands - right and left sides = 14 joint areas)					Max 28
Arthroscopies					2 each
Joint replacements					4 each
SPINE RHEUMATOLOGY					
Clinical spinal pain (2 each for cervical, thoracic, lumbar, sacroiliac)					Max 8
Osteoarticular disease of the spine (4 each for pubic symphysis, ribs, sacroiliac, lumbar, thoracic, cervical)					Max 24
Kyphosis	(Cobb angles) 45-60 >60	 3 6	**Scoliosis**	(Cobb angles) 5-20 21-30 >30	 2 4 6

Sample size

Based on the CHMP advice to use u-HGA24 as the primary efficacy variable but also to show treatment effect on some clinical outcome variables. The sample size calculation was based on the probability to show treatment effect on the AKUSSI score, since very few patients are needed to show treatment effect on HGA levels. Based on data from the previous cross-sectional study of AKU using AKUSSI, and follow-up data, it was calculated that if the measure of efficacy is that nitisinone reduces the mean increase in AKUSSI over the 4-year period to 4 points, compared to the 8 points expected for the control group, and taking the standard deviation of the increase to be 8, then a sample size of 64 per group is required for a two-sided t-test with power 80 % for significance level 0.05. With a 10% drop-out rate, a sample size of 70 per group is required (140 patients in all).

Randomisation

Patients were randomly assigned to one of the two groups in a 1:1 ratio. The randomization was stratified by study center and age (≤ 55 years and > 55 years), since there is evidence that the rate of disease progression increases after an age of approximately 55 years. The study statistician created a program to randomly assign the patients to the two treatment groups using the SAS System. The randomization was carried out by using randomly permuted blocks (4 patients/block) within each study center and age stratum. The randomization was centrally implemented in the electronic CRF system.

Blinding (masking)

During the scientific advice, the CHMP recommended to make the study evaluator blinded as far as possible, e.g. assessments which did not require direct contact between the evaluator and the patient (e.g. evaluation of images) would be blinded during the entire study period. Since, just as for the dose-response study (SONIA 1), the efficacy and safety study (SONIA 2) used objective assessments of efficacy for the primary endpoint (u-HGA24 levels), the Applicant states that the lack of a double-blind design is unlikely to have introduced bias in evaluation of the primary endpoint or those secondary endpoints which were based on serum analyses or evaluator-blinded assessments.

However, the Applicant recognizes that reporting of subjective assessments potentially introduced bias for some of the secondary endpoints, such as pain, QoL assessments, and reporting of AEs.

The majority of the assessments, except for self-reported symptoms such as pain and QoL, were performed by blinded evaluators. Echocardiography (aortic stenosis or sclerosis), scintigraphic scans (osteoarticular disease of joints and spine), X-rays (kyphosis and scoliosis), photographs of eyes and ears (pigmentation), and ear cartilage biopsies were evaluated by completely blinded central assessors separate from the study sites and thus in no contact with study patients or investigators. Objective measurements that were not influenced by the possible knowledge of the patient's treatment were also made by DEXA (osteopenia of the hip) and audiometry. In addition, range of motion and walk tests were assessed by independent site staff without knowledge of patient treatment. Patients were instructed not to tell the hospital staffs about their treatment.

Statistical methods

Based on data from the dose-response study (SONIA 1) it was assumed that u-HGA24 would have a log-normal distribution. The primary analysis was a MMRM as follows: log(u-HGA24) = treatment, site, age category, visit, treatment by- visit interaction where treatment, visit and treatment-by-visit interaction were included as fixed factors while subject-within-site was included as a random factor. A compound symmetry covariance matrix was used. The restricted maximum likelihood method (REML) was used and the degrees of freedom were estimated using Kenwood-Rogers method. Model-based point estimates and associated 95% CIs were calculated. The LS means and confidential intervals (CI) for within-group and between-group estimates were exponentiated, which corresponds to adjusted geometric means and ratio of adjusted geometric means. Data from beyond the 12-month visit were not included in the primary analysis.

For the final analysis of secondary endpoints at month 48, a longitudinal model was fitted for most outcome variables having the fixed factor structure: treatment + site + age category + visit + treatment-by-visit interaction, together with the baseline value as a covariate and with subject-within-site included as a random factor.

No adjustment for multiple comparisons was made.

Following a change in the statistical analysis plan (SAP), the multiple imputation techniques to account for missing data, originally selected, was changed with a tipping point analysis conducted for the following endpoints: u-HGA24, cAKUSSI scores, eye pigmentation, ear pigmentation, osteopenia of the hip, and number of spinal regions with pain.

Imputations were performed in 2 steps. First the intermittently missing observations from both groups were imputed using the Markov-Chain Monte Carlo (MCMC) method assuming missing at random (MAR), generating 500 datasets. This was done by treatment and covariates used in the analysis models (site and age group), resulting in individual imputation models for each treatment*site*age subgroup. In case frequencies in some of the treatment*site*age group subgroups were too small for modelling, the analysis models could be run only by treatment, or by treatment with only one of the covariates (covariate with the smallest p-value in the original analysis was chosen). In the second step, the remaining monotonously missing observations were imputed assuming missing not at random (MNAR) in the nitisinone group, by adding a shift to the MAR imputed value. The shift added in the nitisinone group was calculated as the percentage of the mean value at the given time point in nitisinone group. The monotonously missing observations in the untreated group were imputed assuming MAR. The same analysis models were used for the imputed data as with the original analyses

data. For endpoints modelling the change from baseline, the imputations were performed on the actual values. Changes from baseline were then calculated based on the imputed values. Tipping point results are given for the time points with significant treatment differences in favour of nitisinone.

Results

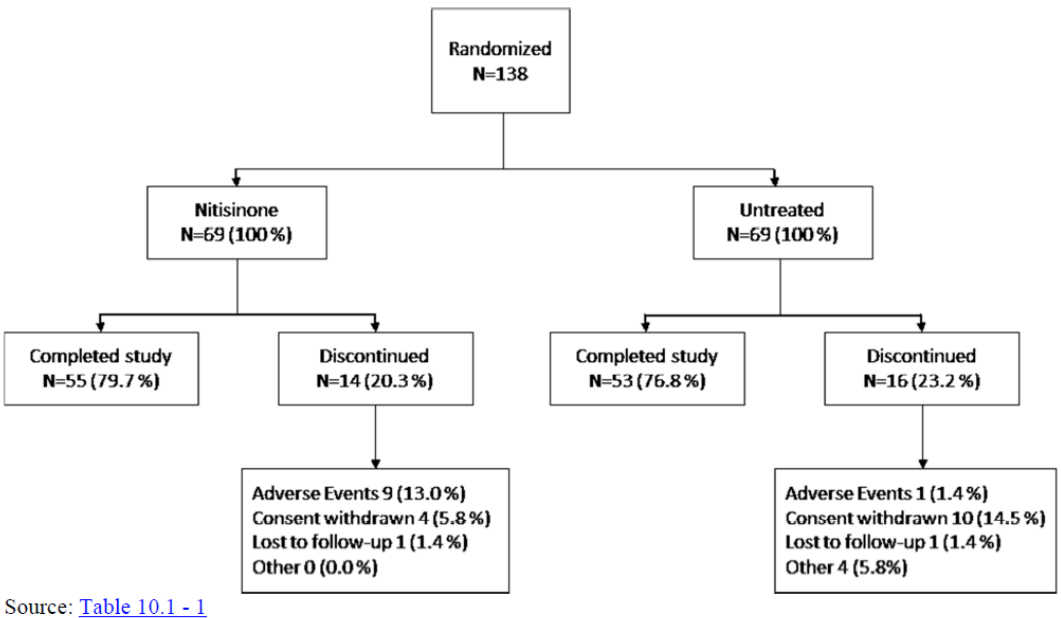
Participant flow

The following table 5 and figure Y show the disposition of patients.

Table 5. Patient disposition (All randomised patients)

	Number of patients (%)		
	Untreated (N=69)	**Nitisinone (N=69)**	**Total (N=138)**
Randomized	69 (100.0)	69 (100.0)	138 (100.0)
Completed	53 (76.8)	55 (79.7)	108 (78.3)
Discontinued before 48-month visit	16 (23.2)	14 (20.3)	30 (21.7)
Primary reason for discontinuation			
Adverse Event	1 (1.4)	9 (13.0)	10 (7.2)
Consent withdrawn	10 (14.5)	4 (5.8)	14 (10.1)
Lost to follow-up	1 (1.4)	1 (1.4)	2 (1.4)
Other	4 (5.8)	0 (0.0)	4 (2.9)

Source: Table 10.1 - 1



Source: Table 10.1 - 1

Figure 7. Patient flow (All randomised patients)

Recruitment

First Subject Screened: May 5, 2014

First Subject Randomized: May 7, 2014

Last Subject's Last Visit: February 15, 2019

Conduct of the study

There were 3 amendments to the original clinical study protocol (CSP).

Amendment no. 1 was approved before the inclusion of any patients in the study. The dose to be used in the study was identified and a number of clarifications regarding the different assessments were made.

Amendment no. 2 introduced some minor changes in some of the study procedures. The amendment also clarified some of the text in the original CSP, a need identified during the initial study conduct.

Amendment no. 3 described some changes to the study staff. MRI was changed from being a secondary to an exploratory assessment, and some further clarifications were made.

Baseline data

Table 6 shows some demographic characteristics of the two groups.

Table 6. Demographic data and baseline characteristics (FAS)

Variable	Statistic	Untreated (N=69)	Nitisinone (N=69)	Total (N=138)
Age (years)	n	69	69	138
	Mean (SD)	47.6 (10.1)	49.0 (11.3)	48.3 (10.7)
	Median (min; max)	48.0 (27; 67)	51.0 (26; 70)	49.0 (26; 70)
Body weight (kg)	n	69	69	138
	Mean (SD)	74.1 (15.6)	74.8 (14.8)	74.4 (15.1)
	Median (min; max)	73.0 (46; 122)	75.0 (36; 110)	74.0 (36; 122)
Height (cm)	n	69	69	138
	Mean (SD)	167 (9.5)	166 (9.2)	167 (9.4)
	Median (min; max)	168 (148; 191)	168 (142; 189)	168 (142; 191)
Sex n (%)	Male	40 (58.0)	45 (65.2)	85 (61.6)
	Female	29 (42.0)	24 (34.8)	53 (38.4)
Race n (%)	White	67 (97.1)	67 (97.1)	134 (97.1)
	Black	0 (0.0)	1 (1.4)	1 (0.7)
	Asian	2 (2.9)	1 (1.4)	3 (2.2)

n: Number of patients observed.
Percentage calculated on N (patients in treatment groups).
Source: Table 10.1 – 4

The age distribution of female is presented in Table 7.

Table 7. age in female patients (FAS)

	Untreated (N = 29)	Nitisinone (N = 24)	Total (N = 53)
Age (years)			
n	29	24	53
Mean (SD)	46.9 (10.50)	51.9 (9.56)	49.2 (10.31)
Median (Range)	48.0 (27 - 67)	54.5 (34 - 70)	50.0 (27 - 70)
Age group (years), n (%)			
≤55 years	20 (69.0)	15 (62.5)	35 (66.0)
>55 years	9 (31.0)	9 (37.5)	18 (34.0)

Source: AGE_FEMFAS_T.SAS 2020-06-24T07:11:02 Z9FRBE

Baseline mean/median scores of the different subdomains of AKUSSI in the overall groups, by sex and age are presented in Tables 8-11

Table 8. Baseline (SD) scores for individual cAKUSSI items by sex (FAS)

	Male						Female					
	Untreated (N = 40)			Nitisinone (N = 45)			Untreated (N = 29)			Nitisinone (N = 24)		
	n	Mean (SD)	Median (Range)	n	Mean (SD)	Median (Range)	n	Mean (SD)	Median (Range)	n	Mean (SD)	Median (Range)
Non-spine, non-rheumatology	40	47.3 (20.22)	46.0 (6 - 97)	45	50.2 (22.44)	43.0 (12 - 94)	29	33.0 (20.16)	33.0 (0 - 81)	24	41.5 (20.79)	41.0 (9 - 82)
Eye pigmentation	40	15.8 (9.71)	16.0 (0 - 32)	45	17.3 (8.90)	16.0 (0 - 32)	29	11.7 (9.07)	12.0 (0 - 32)	24	17.2 (9.83)	16.0 (0 - 32)
Ear pigmentation	40	4.2 (2.71)	4.0 (0 - 8)	45	4.2 (2.89)	4.0 (0 - 8)	29	3.5 (3.05)	4.0 (0 - 8)	24	4.1 (3.09)	4.0 (0 - 8)
Prostate stones[a]	39	2.2 (2.57)	0.0 (0 - 8)	45	2.5 (2.74)	4.0 (0 - 8)	0			0		
Renal stones	39	2.2 (4.19)	0.0 (0 - 16)	45	3.6 (6.08)	0.0 (0 - 24)	29	1.1 (2.37)	0.0 (0 - 8)	24	1.2 (3.43)	0.0 (0 - 16)
Osteopenia of the hip	37	2.2 (2.02)	2.0 (0 - 6)	45	2.2 (2.31)	2.0 (0 - 6)	27	2.1 (2.11)	2.0 (0 - 6)	23	2.3 (2.23)	2.0 (0 - 6)
Adult fractures	40	2.6 (7.55)	0.0 (0 - 40)	45	2.5 (5.35)	0.0 (0 - 24)	29	2.2 (7.68)	0.0 (0 - 40)	24	2.3 (5.52)	0.0 (0 - 24)
Ruptures	40	5.2 (7.59)	0.0 (0 - 24)	45	3.6 (8.28)	0.0 (0 - 48)	29	1.4 (3.75)	0.0 (0 - 16)	24	2.3 (6.00)	0.0 (0 - 24)
Aortic valve stenosis/sclerosis	40	1.6 (3.05)	0.0 (0 - 10)	45	2.3 (3.45)	0.0 (0 - 12)	29	1.8 (3.31)	0.0 (0 - 12)	24	2.3 (3.85)	0.0 (0 - 12)
Hearing impairment	40	2.9 (2.31)	3.0 (0 - 8)	45	2.8 (2.38)	3.0 (0 - 8)	29	1.7 (1.63)	1.0 (0 - 5)	23	1.7 (1.75)	1.0 (0 - 6)
Dark eardrum	39	9.1 (4.94)	12.0 (0 - 12)	44	9.4 (4.74)	12.0 (0 - 12)	29	7.7 (5.76)	12.0 (0 - 12)	23	8.6 (5.06)	12.0 (0 - 12)
Non-spine rheumatology	40	21.1 (8.52)	20.5 (6 - 45)	45	20.9 (11.12)	20.0 (0 - 43)	29	18.6 (9.43)	17.0 (2 - 43)	24	16.7 (7.88)	16.5 (4 - 31)
Clinical joint pain	40	4.5 (3.20)	4.0 (0 - 11)	45	5.0 (3.00)	5.0 (0 - 11)	29	4.8 (3.37)	5.0 (0 - 13)	24	4.3 (3.05)	4.0 (0 - 12)
None-spine osteoarticular disease	40	14.9 (5.80)	16.0 (4 - 28)	43	13.5 (6.32)	14.0 (4 - 28)	29	11.4 (6.85)	12.0 (0 - 24)	24	10.4 (5.81)	12.0 (0 - 20)
Arthroscopies	40	0.6 (1.11)	0.0 (0 - 4)	44	0.9 (1.69)	0.0 (0 - 6)	29	0.3 (0.94)	0.0 (0 - 4)	24	0.2 (0.56)	0.0 (0 - 2)
Joint replacements	40	1.1 (3.26)	0.0 (0 - 16)	45	2.1 (4.23)	0.0 (0 - 16)	29	2.1 (5.30)	0.0 (0 - 24)	24	1.8 (3.73)	0.0 (0 - 12)
Spine rheumatology	40	18.8 (8.71)	19.5 (4 - 38)	45	19.9 (10.27)	20.0 (2 - 34)	29	19.8 (10.61)	20.0 (2 - 37)	24	21.7 (9.12)	23.0 (4 - 37)
Clinical spinal pain	40	4.3 (2.29)	4.0 (0 - 8)	45	4.4 (2.66)	4.0 (0 - 8)	29	5.2 (2.48)	6.0 (0 - 8)	24	5.0 (2.77)	5.0 (0 - 8)
Osteoarticular disease of the spine	40	12.1 (8.13)	12.0 (0 - 24)	43	13.7 (8.61)	16.0 (0 - 24)	29	11.9 (9.16)	12.0 (0 - 24)	24	13.5 (8.57)	14.0 (0 - 24)
Kyphosis	40	0.3 (0.91)	0.0 (0 - 3)	45	0.5 (1.32)	0.0 (0 - 6)	29	0.7 (1.53)	0.0 (0 - 6)	24	1.0 (1.44)	0.0 (0 - 3)
Scoliosis	40	2.1 (0.71)	2.0 (0 - 6)	45	1.9 (0.50)	2.0 (0 - 2)	29	2.0 (0.53)	2.0 (0 - 4)	24	2.2 (1.01)	2.0 (0 - 6)

Source: CAKUSITM_SEX_FAS_T.SAS 2020-06-24T07:10:23 Z9FRBE
[a] Male only

Table 9. Baseline (SD) scores for individual cAKUSSI items by age (FAS)

	≤ 55 years						> 55 years					
	Untreated (N = 50)			Nitisinone (N = 46)			Untreated (N = 19)			Nitisinone (N = 23)		
	n	Mean (SD)	Median (Range)	n	Mean (SD)	Median (Range)	n	Mean (SD)	Median (Range)	n	Mean (SD)	Median (Range)
Non-spine, non-rheumatology	50	35.8 (20.62)	36.0 (0 - 97)	46	40.5 (20.00)	37.0 (9 - 88)	19	55.7 (15.83)	54.0 (24 - 94)	23	60.6 (20.34)	65.0 (14 - 94)
Eye pigmentation	50	11.3 (7.91)	12.0 (0 - 28)	46	13.8 (6.84)	16.0 (0 - 32)	19	21.5 (9.91)	24.0 (0 - 32)	23	24.2 (9.46)	28.0 (0 - 32)
Ear pigmentation	50	3.5 (2.94)	4.0 (0 - 8)	46	3.8 (2.93)	4.0 (0 - 8)	19	5.1 (2.34)	4.0 (0 - 8)	23	4.9 (2.88)	6.0 (0 - 8)
Prostate stones[a]	29	2.3 (2.73)	0.0 (0 - 8)	31	2.3 (2.88)	0.0 (0 - 8)	10	1.6 (2.07)	0.0 (0 - 4)	14	2.9 (2.44)	4.0 (0 - 8)
Renal stones	49	1.8 (3.75)	0.0 (0 - 16)	46	2.8 (5.65)	0.0 (0 - 24)	19	1.5 (3.04)	0.0 (0 - 12)	23	2.8 (5.04)	0.0 (0 - 20)
Osteopenia of the hip	49	1.8 (1.76)	2.0 (0 - 6)	46	1.7 (2.13)	1.0 (0 - 6)	15	3.3 (2.47)	2.0 (0 - 6)	22	3.4 (2.17)	3.0 (0 - 6)
Adult fractures	50	3.0 (8.69)	0.0 (0 - 40)	46	2.1 (5.45)	0.0 (0 - 24)	19	0.8 (2.52)	0.0 (0 - 8)	23	3.1 (5.25)	0.0 (0 - 16)
Ruptures	50	2.2 (4.86)	0.0 (0 - 24)	46	2.4 (5.02)	0.0 (0 - 24)	19	7.2 (8.80)	8.0 (0 - 24)	23	4.5 (11.01)	0.0 (0 - 48)
Aortic valve stenosis/sclerosis	50	0.9 (2.33)	0.0 (0 - 10)	46	1.3 (3.16)	0.0 (0 - 12)	19	3.6 (4.14)	4.0 (0 - 12)	23	4.2 (3.66)	4.0 (0 - 12)
Hearing impairment	50	2.0 (2.01)	2.0 (0 - 8)	46	2.0 (2.07)	1.5 (0 - 8)	19	3.5 (2.12)	4.0 (0 - 8)	22	3.2 (2.43)	3.0 (0 - 8)
Dark eardrum	50	8.0 (5.64)	12.0 (0 - 12)	46	8.9 (5.18)	12.0 (0 - 12)	18	9.7 (4.19)	12.0 (0 - 12)	21	9.7 (4.01)	12.0 (0 - 12)
Non-spine rheumatology	50	18.2 (8.03)	17.5 (2 - 39)	46	17.6 (10.89)	17.5 (0 - 43)	19	24.9 (9.54)	24.0 (8 - 45)	23	23.2 (7.79)	21.0 (14 - 42)
Clinical joint pain	50	4.1 (3.35)	3.0 (0 - 13)	46	4.1 (3.00)	4.0 (0 - 12)	19	5.9 (2.61)	6.0 (1 - 11)	23	6.1 (2.62)	6.0 (0 - 11)
None-spine osteoarticular disease	50	12.8 (6.55)	14.0 (0 - 24)	44	12.4 (7.01)	12.0 (0 - 28)	19	14.9 (6.09)	16.0 (4 - 28)	23	12.5 (4.72)	12.0 (2 - 24)
Arthroscopies	50	0.5 (1.03)	0.0 (0 - 4)	46	0.7 (1.52)	0.0 (0 - 6)	19	0.4 (1.07)	0.0 (0 - 4)	22	0.5 (1.26)	0.0 (0 - 4)
Joint replacements	50	0.7 (3.59)	0.0 (0 - 24)	46	1.0 (2.72)	0.0 (0 - 12)	19	3.6 (5.15)	0.0 (0 - 16)	23	4.0 (5.39)	0.0 (0 - 16)
Spine rheumatology	50	17.0 (9.20)	16.0 (2 - 32)	46	17.5 (9.56)	18.0 (2 - 34)	19	24.9 (7.82)	26.0 (12 - 38)	23	26.7 (7.37)	28.0 (10 - 37)
Clinical spinal pain	50	4.1 (2.23)	4.0 (0 - 8)	46	4.0 (2.75)	4.0 (0 - 8)	19	6.1 (2.26)	6.0 (2 - 8)	23	5.8 (2.17)	6.0 (2 - 8)
Osteoarticular disease of the spine	50	10.3 (8.59)	8.0 (0 - 24)	44	11.3 (8.68)	12.0 (0 - 24)	19	16.4 (6.65)	16.0 (4 - 24)	23	18.1 (6.25)	20.0 (0 - 24)
Kyphosis	50	0.5 (1.31)	0.0 (0 - 6)	46	0.7 (1.44)	0.0 (0 - 6)	19	0.3 (0.95)	0.0 (0 - 3)	23	0.7 (1.27)	0.0 (0 - 3)
Scoliosis	50	2.0 (0.40)	2.0 (0 - 4)	46	1.9 (0.59)	2.0 (0 - 4)	19	2.1 (1.05)	2.0 (0 - 6)	23	2.1 (0.95)	2.0 (0 - 6)

Source: CAKUSITM_AGE_FAS_T.SAS 2020-06-24T07:10:21 Z9FRBE
a Male only

Table 10. Change from baseline in cAKUSSI scores by sex (FAS)

		Males		Females	
Visit	**Statistic**	**Untreated (N=40)**	**Nitisinone (N=45)**	**Untreated (N=29)**	**Nitisinone (N=24)**
Baseline	n	40	45	29	24
	Mean	87.1	90.9	71.3	79.8
	SD	31.2	35.1	34.7	31.9
	Median	86.5	97.0	73.0	82.5
	min	30	16	14	25
	max	165	152	138	133
Month 12	Adjusted mean	3.7	0.1	0.0	-1.0
	95% CI	0.2; 7.2	-3.0; 3.2	-3.7; 3.8	-5.0; 3.1
	Adjusted mean (difference nitisinone-untreated)		-3.5		-1.0
	95% CI		-7.7; 0.6		-6.2; 4.2
	p-value*		0.090		0.697

Month 24	Adjusted mean	8.9	7.3	2.8	3.7
	95% CI	4.1; 13.8	3.0; 11.7	-3.3; 9.0	-3.2; 10.6
	Adjusted mean (difference nitisinone-untreated)		-1.6		0.8
	95% CI		-7.7; 4.6		-8.2; 9.9
	p-value*		0.613		0.853
Month 36	Adjusted mean	12.6	8.1	10.0	1.9
	95% CI	6.6; 18.7	2.5; 13.8	2.9; 17.1	-6.2; 10.1
	Adjusted mean (difference nitisinone-untreated)		-4.5		-8.1
	95% CI		-12.5; 3.5		-18.8; 2.6
	p-value*		0.267		0.134
Month 48	Adjusted mean	19.0	11.0	12.9	1.3
	95% CI	12.7; 25.4	5.3; 16.8	3.5; 22.3	-9.5; 12.0
	Adjusted mean (difference nitisinone-untreated)		-8.0		-11.6
	95% CI		-16.3; 0.4		-25.8; 2.5
	p-value*		0.060		0.105

*Change from baseline is analyzed using a mixed model with repeated measurements (MMRM).
Created using akussi1.sas, akussi2.sas, akussi3.sas and rand.sas version 1 in S:\Statistical Documents\AKU\Trials\SONIA 2\Final analysis\Final programming\Data by EP on 18MAY20:13:23:55.

Table 11. Change from baseline in mAKUSSI scores by sex (FAS)

		Males		**Females**	
Visit	**Statistic**	**Untreated (N=40)**	**Nitisinone (N=45)**	**Untreated (N=29)**	**Nitisinone (N=24)**
Baseline	n	40	45	29	24
	Mean	58.3	60.2	48.4	50.3
	SD	25.1	28.8	24.0	21.3
	Median	55.5	61.0	49.0	49.0
	min	21	4	9	9
	max	117	113	101	92
Month 12	Adjusted mean	3.9	2.9	0.2	0.0
	95% CI	1.7; 6.1	1.0; 4.8	-1.9; 2.4	-2.3; 2.2
	Adjusted mean (difference nitisinone-untreated)		-1.0		-0.3
	95% CI		-3.6; 1.6		-3.3; 2.7
	p-value*		0.444		0.852

Month 24	Adjusted mean	8.4	7.9	2.0	3.3
	95% CI	4.3; 12.4	4.3; 11.6	-3.2; 7.3	-2.6; 9.3
	Adjusted mean (difference nitisinone-untreated)		-0.5		1.3
	95% CI		-5.7; 4.8		-6.5; 9.2
	p-value*		0.863		0.736
Month 36	Adjusted mean	11.6	9.2	4.6	2.3
	95% CI	6.5; 16.6	4.5; 13.9	-1.6; 10.8	-4.8; 9.4
	Adjusted mean (difference nitisinone-untreated)		-2.4		-2.3
	95% CI		-9.1; 4.4		-11.7; 7.1
	p-value*		0.484		0.625
Month 48	Adjusted mean	16.1	12.0	8.1	3.4
	95% CI	10.8; 21.3	7.2; 16.8	0.8; 15.3	-4.9; 11.7
	Adjusted mean (difference nitisinone-untreated)		-4.1		-4.7
	95% CI		-11.1; 2.9		-15.7; 6.4
	p-value*		0.251		0.399

*Change from baseline is analyzed using a mixed model with repeated measurements (MMRM).
Created using akussi1.sas, akussi2.sas, akussi3.sas and rand.sas version 1 in S:\Statistical Documents\AKU\Trials\SONIA 2\Final analysis\Final programming\Data by EP on 18MAY20:13:23:59.

Distribution of patients in categories based on baseline mAKUSSI score are presented in Table 12.

Table 12. Baseline mAKUSSI scores, categorised (FAS)

Table 3	Baseline mAKUSSI scores, categorized (Full analysis set)	
mAKUSSI scores category	**Untreated (N = 69)**	**Nitisinone (N = 69)**
0 - <5	0	1 (1.4)
5 - <10	1 (1.4)	1 (1.4)
10 - <20	3 (4.3)	5 (7.2)
20 - <30	8 (11.6)	5 (7.2)
30 - <40	8 (11.6)	6 (8.7)
40 - <50	12 (17.4)	9 (13.0)
≥50	37 (53.6)	42 (60.9)

Source: MAKUS_CAT_FAS_T.SAS 2020-06-24T07:10:18 Z9FRBE

Baseline characteristics regarding AKU specific questions are presented in Table 13.

Table 13. Medical history based on AKU specific questions registered as baseline efficacy data in SONIA -2 (FAS).

	Number (%) of patients	
	Untreated (N=69)	**Nitisinone (N=69)**
Prostate stones	18 (45.0)[a]	23 (51.1)[a]
Renal stones	18 (26.1)	22 (31.9)
Osteopenia of the hip[b]	42 (60.9)	46 (66.7)
Fracture[c]	11 (15.9)	15 (21.7)
Ruptures (tendon, ligament, muscle)	21 (30.4)	17 (24.6)
Aortic stenosis and sclerosis	18 (26.1)	25 (36.2)
Kyphosis[d]	10 (14.5)	15 (21.7)

	Number (%) of patients		
	Untreated (N=69)	**Nitisinone (N=69)**	**Data source CSR SONIA 2**
Scoliosis[e]	67 (97.1)	65 (94.2)	Table 10.2.2.1-45
Arthroscopies	13 (18.8)	13 (18.8)	Table 10.2.2.1-48
Joint replacements	12 (17.4)	17 (24.6)	Table 10.2.2.1-50

Percentage calculated on N.
[a] Percentage calculated on male patients; N=40 in the untreated group and N=45 in the nitisinone group.
[b] Patients with osteopenia or osteoporosis.
[c] Adult fracture (fracture occurring from age 18 or later.
[d] Cobbs angel of 45 or more.
[e] Degree of scoliosis of 5 or more.
Abbreviations: N, number of observed patients.

Table 14 shows the use of other medications by the patients in the two groups.

Table 14. Most commonly (≥5% of patients (used concomitant medication ongoing at randomisation by ATC class (FAS)

ATC Class	Number of patients (%)		
	Untreated (N=69)	Nitisinone (N=69)	Total (N=138)
Anilides	16 (23.2)	14 (20.3)	30 (21.7)
Proton pump inhibitors	13 (18.8)	14 (20.3)	27 (19.6)
Vitamin D and analogues	8 (11.6)	16 (23.2)	24 (17.4)
Acetic acid derivatives and related substances	11 (15.9)	11 (15.9)	22 (15.9)
Beta blocking agents, selective	14 (20.3)	8 (11.6)	22 (15.9)
Propionic acid derivatives	7 (10.1)	11 (15.9)	18 (13.0)
Ascorbic acid (vitamin C), plain	9 (13.0)	8 (11.6)	17 (12.3)
Other opioids	8 (11.6)	8 (11.6)	16 (11.6)
ACE inhibitors, plain	7 (10.1)	7 (10.1)	14 (10.1)
HMG CoA reductase inhibitors	6 (8.7)	6 (8.7)	12 (8.7)
Benzodiazepine derivatives	3 (4.3)	7 (10.1)	10 (7.2)
Other antiepileptics	5 (7.2)	4 (5.8)	9 (6.5)
Salicylic acid and derivatives	2 (2.9)	7 (10.1)	9 (6.5)
Dihydropyridine derivatives	3 (4.3)	5 (7.2)	8 (5.8)
Other antiinflammatory and antirheumatic agents, non-steroids	3 (4.3)	5 (7.2)	8 (5.8)
Angiotensin II antagonists, plain	3 (4.3)	4 (5.8)	7 (5.1)
Calcium	4 (5.8)	3 (4.3)	7 (5.1)
Coxibs	4 (5.8)	3 (4.3)	7 (5.1)

Percentage calculated on N (patients in treatment groups)
Source: Table 10.1 – 7

Medications that were started after the randomizations by the patients enrolled are presented in Table 15

Table 15. Concomitant medications, onset of new medications after randomisation (FAS)

		Number of patients (%)		
ATC Class	**WHO term**	**Untreated (N=69)**	**Nitisinone (N=69)**	**Total (N=138)**
Anilides	Total	19 (27.5)	20 (29.0)	39 (28.3)
	Paracetamol	16 (23.2)	17 (24.6)	33 (23.9)
	Paracetamol, combinations excl. psycholeptics	3 (4.3)	2 (2.9)	5 (3.6)
	Paracetamol, combinations with psycholeptics	0 (0.0)	1 (1.4)	1 (0.7)
Acetic acid derivatives and related substances	Total	10 (14.5)	19 (27.5)	29 (21.0)
	Diclofenac	9 (13.0)	12 (17.4)	21 (15.2)
	Diclofenac, combinations	0 (0.0)	5 (7.2)	5 (3.6)
	Ketorolac	1 (1.4)	1 (1.4)	2 (1.4)
	Aceclofenac	0 (0.0)	1 (1.4)	1 (0.7)
Propionic acid derivatives	Total	12 (17.4)	17 (24.6)	29 (21.0)
	Ibuprofen	5 (7.2)	7 (10.1)	12 (8.7)
	Ketoprofen	5 (7.2)	5 (7.2)	10 (7.2)
	Naproxen	2 (2.9)	3 (4.3)	5 (3.6)
	Naproxen and esomeprazole	0 (0.0)	1 (1.4)	1 (0.7)
	Tiaprofenic acid	0 (0.0)	1 (1.4)	1 (0.7)
Bisphosphonates	Total	5 (7.2)	9 (13.0)	14 (10.1)
	Risedronic acid	3 (4.3)	5 (7.2)	8 (5.8)
	Alendronic acid	1 (1.4)	4 (5.8)	5 (3.6)
	Ibandronic acid	1 (1.4)	0 (0.0)	1 (0.7)

Data indicate that more nitisinone-treated than untreated patients were prescribed analgesic medication after randomization. However, the collection of concomitant medication data was not set up to quantify the use of different drugs. After the study had been ongoing for a couple of years, the site in Paris wanted to compare the use of analgesics, as several patients on nitisinone claimed they had reduced their use considerably. But with only information about start and stop dates and varying degrees of strength and dosing information (very often stated as "on demand"), this was not possible.

Numbers analysed

Populations for analysis and data on treatment compliance are shown in Tables 16 and 17.

Table 16. Summary of analysis sets (all randomised patients)

	Number (%) of patients		
	Untreated (N = 69)	Nitisinone (N = 69)	Total (N = 138)
Patients randomized	69 (100.0)	69 (100.0)	138 (100.0)
Patients in Safety analysis set	69 (100.0)	69 (100.0)	138 (100.0)
Patients in Full analysis set (FAS)	69 (100.0)	69 (100.0)	138 (100.0)
Patients in Per protocol set (PP)	63 (91.3)	66 (95.7)	129 (93.5)

Source: Table 10.1 - 2

Table 17. Treatment compliance (FAS, nitisinone- treated only)

Visit	% of planned consumption	Nitisinone (N=69)
Month 12	n	69
	≥ 80%, n (%)	69 (100.0)
	< 80%, n (%)	0 (0.0)
Month 24	n	66
	≥ 80%, n (%)	66 (95.7)
	< 80%, n (%)	0 (0.0)
Month 36	n	62
	≥ 80%, n (%)	62 (89.9)
	< 80%, n (%)	0 (0.0)
Month 48	n	59
	≥ 80%, n (%)	59 (85.5)
	< 80%, n (%)	0 (0.0)

n: Number of patients observed
Percentage of patients calculated on N (patients in treatment groups)
Source: Table 10.1 - 9

Moreover, 4 subjects had low or undetectable levels of serum nitisinone at one of the planned visits. Very few patients were discovered to have low levels of serum nitisinone despite reporting to have taken up the drug correctly. It is unlikely that such small numbers had some impact on the general reliability of the results.

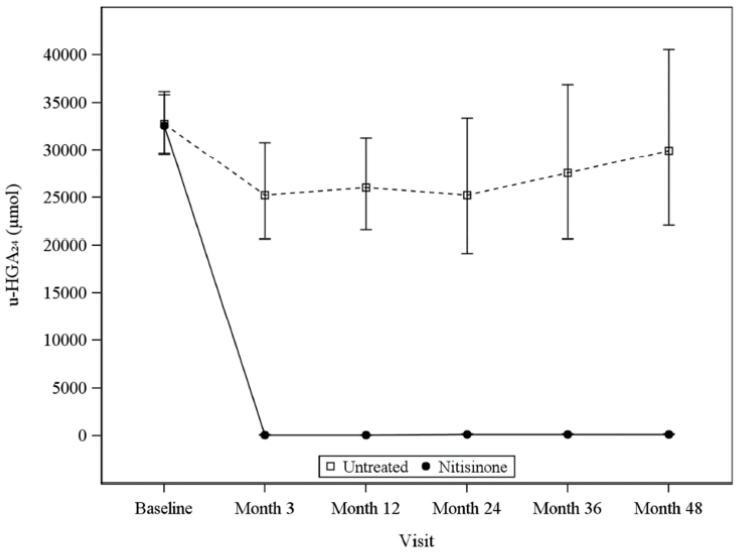
Outcomes and estimation

The results of the MMRM for u-HGA24 at baseline, Months 12 and 48 are presented in table 18 and Figure 8.

Table 18. U-HGA24 (µmol) at baseline and Months 12 and 48 (FAS)

Visit	Statistic	Untreated (N=69)	Nitisinone (N=69)
Baseline	n	69	69
	Mean (SD)	35393.6 (13868.5)	35018.7 (13124.3)
	Geometric mean	32746.1	32572.5
Month 12	Adjusted geometric mean	26027.9	85.7
	95% CI	21649.6; 31291.8	71.8; 102.2
	Adjusted geometric mean (ratio nitisinone/untreated)	0.003	
	95% CI	0.003; 0.004	
	p-value	<0.001	
Month 48	Adjusted geometric mean	29936.1	158.1
	95% CI	22090.6; 40568.0	117.0; 213.6
	Adjusted geometric mean (ratio nitisinone/untreated)	0.005	
	95% CI	0.003; 0.008	
	p-value	<0.001	

Source: Table 10.2.1 - 2



Graph shows geometric mean (95% CI) for baseline and adjusted geometric mean (95% CI) for later time points.
Source: Figure 10.2.1 – 1

Figure 8. U-HGA24 (µmol) over time (FAS)

Nitisinone reduced u-HGA24 by 99.7% compared to no treatment. The maintenance of this effect was observed over the whole duration of the study, ie 4 years of treatment.

The occurrence of achieved target level (<300 µmol) of u-HGA24 at 12 and 48 months are presented in Table 19.

Table 19. Number of patients with u-HGA24 below and above the target level (300 µmol) at Months 12 and 48 (FAS)

Visit	$U\text{-}HGA_{24}$	Untreated (N=69) n (%)	Nitisinone (N=69) n (%)
Month 12	< 300 µmol (%)	0 (0.0)	61 (88.4)
	≥ 300 µmol (%)	69 (100.0)	8 (11.6)
	p- value		< 0.001
Month 48	< 300 µmol (%)	0 (0.0)	41 (59.4)
	≥ 300 µmol (%)	69 (100.0)	28 (40.6)
	p- value		< 0.001

n: Number of patients observed
Percentage calculated on N (patients in treatment groups)
Data for withdrawn patients, and other patients with missing data, have been set to ≥ 300 µmol
Source: Table 10.2.1 - 8

Treatment effect related to switching to 2 mg is presented in Table 20 below.

Table 20. u-HGA24 (µmol) at any time during the study, in 8 patients who decreased the nitisinone dose to 1 mg/day

Statistic	Nitisinone 10 mg	Nitisinone 2 mg
n	18	12
Mean (SD)	154.5 (121.7)	1586.2 (953.3)
Median (min; max)	132.0 (18.7; 443.5)	1298.3 (690.0; 4064.6)

n: Number of serum samples
Samples from patients with s-nitisinone <0.2 have been omitted
Source: Table 10.2.1 - 7

During the treatment with nitisinone 10 mg, some patients developed keratopathy and the dose were reduced to 2 mg/kg. The levels of u-HGA 24h in this subgroup showed an increase of about 10 fold in u-HGA after switching to nitisinone 2 mg.

cAKUSSI scores at baseline and Month 48 are shown in the table 21.

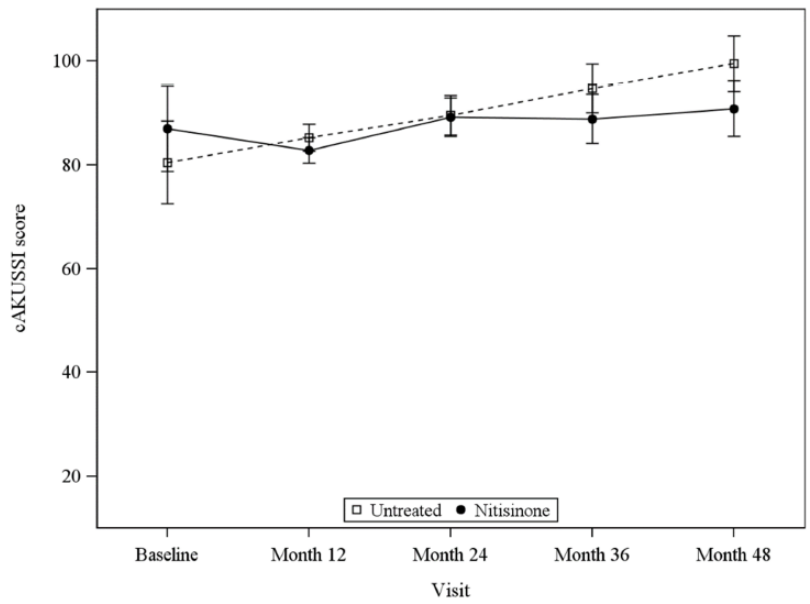
Table 21. cAKUSSI score at baseline and Month 48 (FAS)

		Untreated (N=69)		Nitisinone (N=69)	
Visit	**Statistic**	**Value**	**Change from baseline**	**Value**	**Change from baseline**
Baseline	n	69		69	
	Mean (SD)	80.5 (33.4)		87.0 (34.2)	
	Median (min; max)	82.0 (14; 165))		91.0 (16; 152)	
Month 48	Adjusted mean		16.1		7.4
	95% CI		10.7; 21.4		2.1; 12.8
	Adjusted mean (difference nitisinone-untreated				-8.6
	95% CI				-16.0; -1.2
	p-value*				0.023

n: Number of patients observed
Source: Table 10.2.2 – 1, Table 10.2.2 – 2

The more severe are the manifestations of AKU, the higher the score is. A reduction of 8.6 points was observed in the nitisinone group compared to untreated (95% CI: -16.0, -1.2; p=0.023). The difference between the two groups in the change from baseline to Month 48 was statistically significant. The Applicant stated that "a tipping point analysis was also conducted to assess the potential impact of missing data. While it seems that missing data could have an impact on the significant outcome, the continuous improvement over time until Month 36, after which the majority of missing data occurs, indicates that the statistically significant outcome at Month 48 is true."

The trend for cAKUSSI along the duration of the study is presented in Figure 9.



Source: Figure 10.2.2 - 1

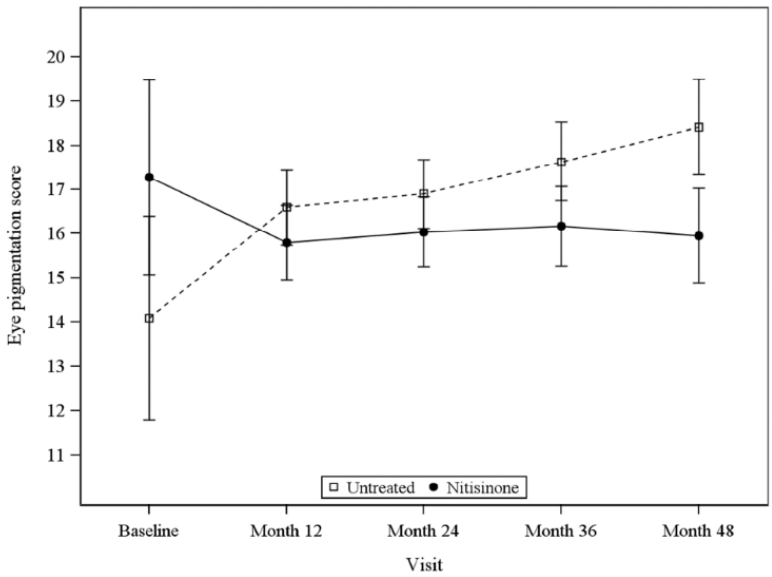
Figure 9. Adjusted mean and 95% CI for cAKUSSI scores over time (FAS)

The following table 22 and figure 10 show the component of **cAKUSSI** regarding eye pigmentation.

Table 22. Total eye pigmentation scores at baseline and Month 48

		Untreated (N=69)		Nitisinone (N=69)	
Visit	**Statistic**	**Value**	**Change from baseline**	**Value**	**Change from baseline**
Baseline	n	69		69	
	Mean (SD)	14.1 (9.6)		17.3 (9.2)	
	Median (min; max)	16.0 (0; 32)		16.0 (0; 32)	
Month 48	Adjusted mean		3.0		0.5
	95% CI		1.9; 4.1		-0.5; 1.6
	Adjusted mean (difference nitisinone-untreated)				-2.5
	95% CI				-3.9; -1.0
	p-value				0.001

n: Number of patients observed
Source: Table 10.2.2.1 – 1, Table 10.2.2.1 – 2



Source: Figure 10.2.2.1 -1

Figure 10. Adjusted mean and 95%CI for eye pigmentation scores over time (FAS)

The following table 23 shows the baseline and yearly cAKUSSI scores by study site.

Table 23. Change from baseline in cAKUSSI scores by site (FAS)

Visit	Statistic	Liverpool Untreated (N=21)	Liverpool Nitisinone (N=20)	Piešťany Untreated (N=32)	Piešťany Nitisinone (N=33)	Paris Untreated (N=16)	Paris Nitisinone (N=16)
Baseline	n	21	20	32	33	16	16
	Mean	99.1	96.9	70.8	74.7	75.4	100.1
	SD	39.1	36.6	28.3	34.0	25.6	22.2
	Median	107.0	102.0	72.5	73.0	76.0	106.0
	min	17	34	14	16	23	51
	max	165	152	126	144	138	132
Month 12	Adjusted mean	3.6	-2.5	-3.7	-3.3	7.7	1.5
	95% CI	-1.4; 8.6	-7.0; 2.0	-7.0; -0.4	-6.6; 0.0	1.3; 14.1	-4.3; 7.3
	Adjusted mean (difference nitisinone-untreated)		-6.1		0.4		-6.2
	95% CI		-12.4; 0.3		-3.9; 4.7		-15.0; 2.7
	p-value*		0.063		0.844		0.164
Month 24	Adjusted mean	8.2	-0.7	-2.3	4.1	17.8	11.3
	95% CI	2.6; 13.8	-6.0; 4.7	-7.8; 3.3	-1.5; 9.8	9.3; 26.2	3.6; 18.9
	Adjusted mean (difference nitisinone-untreated)		-8.9		6.4		-6.5
	95% CI		-16.4; -1.4		-1.3; 14.1		-18.0; 5.0
	p-value*		0.021		0.102		0.257
Month 36	Adjusted mean	11.8	-2.2	2.4	2.6	25.7	13.9
	95% CI	5.6; 17.9	-8.6; 4.1	-5.4; 10.2	-5.3; 10.6	16.2; 35.3	5.4; 22.5
	Adjusted mean (difference nitisinone-untreated)		-14.0		0.2		-11.8
	95% CI		-22.7; -5.3		-10.8; 11.2		-24.8; 1.2
	p-value*		0.002		0.969		0.074
Month 48	Adjusted mean	14.8	1.8	8.6	3.6	29.0	16.1
	95% CI	8.2; 21.4	-4.9; 8.5	-0.7; 17.8	-5.6; 12.8	17.5; 40.4	5.8; 26.4
	Adjusted mean (difference nitisinone-untreated)		-13.0		-4.9		-12.9
	95% CI		-22.2; -3.8		-17.9; 8.0		-28.4; 2.6
	p-value*		0.007		0.447		0.099

*Change from baseline is analyzed using a mixed model with repeated measurements (MMRM).
Created using akussi1.sas, akussi2.sas, akussi3.sas and rand.sas version 1 in S:\Statistical Documents\AKU\Trials\SONIA 2\Final analysis\Final programming\Data EP on 12DEC19:10:11:02.

mAKUSSI scores at baseline and Month 48 are shown in the table 24.

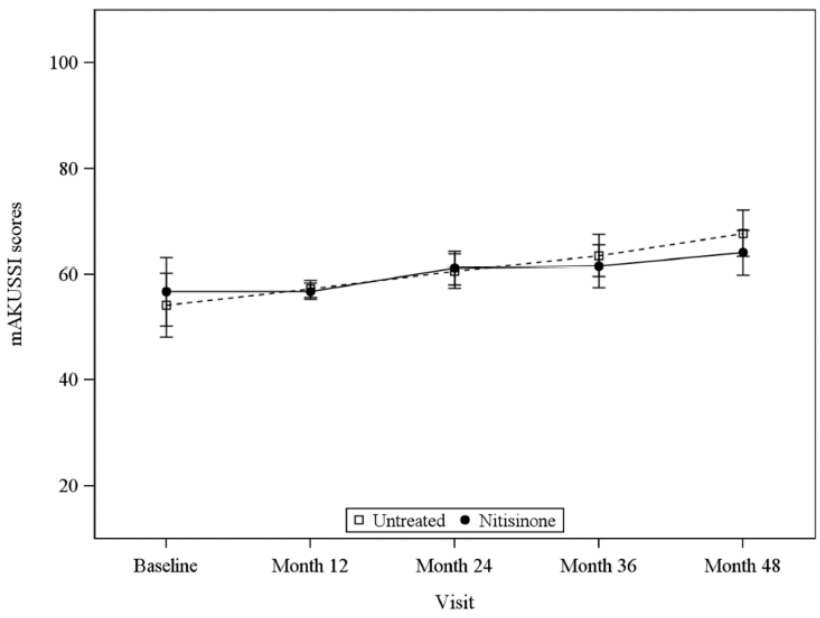
Table 24 mAKUSSI score at baseline and Month 48 (FAS)

		Untreated (N=69)		Nitisinone (N=69)	
Visit	**Statistic**	**Value**	**Change from baseline**	**Value**	**Change from baseline**
Baseline	n	69		69	
	Mean (SD)	54.1 (24.9)		56.7 (26.7)	
	Median (min; max)	52.0 (9; 117)		59.0 (4; 113)	
Month 48	Adjusted mean		12.4		8.8
	95% CI		8.1; 16.7		4.5; 13.1
	Adjusted mean (difference nitisinone-untreated)				3.6
	95% CI				-9.6; 2.4
	p-value*				0.234

n: Number of patients observed
Source: Table 10.2.2 – 6, Table 10.2.2 – 7

At Month 48 there was no notable difference between the two groups in change from baseline. Erratum: a minus sign "-" should be added before "3.6" in the difference between nitisinone and untreated group).

The trend for cAKUSSI over the duration of the study is presented in Figure 11.



Source: Figure 10.2.2 - 2

Figure 11. Adjusted mean and 95% CI for mAKUSSI scores over time (FAS)

A continuous increase in mAKUSSI in the untreated group from baseline to Month 48 was reported, while a slower increase was observed for the nitisinone group.

Results of main individual variables of mAKUSSI score (prostate and renal stones; osteopenia of the hips; adult fractures; ruptures of tendon, ligament of muscle; aortic sclerosis and stenosis; pains of the joints, spine; athroscopies; osteoarticular disease,; joint replacements, scoliosis) are presented in Tables 25-34 and Figures 12-15.

Table 25. Cumulative number of prostate stone episodes reported yearly (FAS, male patients)

Visit	Number (%) of patients reporting at least one episode		Number of episodes reported	
	Untreated (N=40)	Nitisinone (N=45)	Untreated (N=40)	Nitisinone (N=45)
Baseline	18 (45.0)	23 (51.1)	21	28
New episodes since baseline				
Month 12	14 (35.0)	11 (24.4)	15	13
Month 24	17 (42.5)	12 (26.7)	22	16
Month 36	18 (45.0)	15 (33.3)	23	19
Month 48	19 (47.5)	15 (33.3)	24	24

Percentage calculated on N. Missing episodes are counted as no episodes.
Source: Table 10.2.2.1 – 6

There was no difference in prostate stones episodes prevalence at 48 months between the two groups (p=0.268).

Table 26. Cumulative number of renal stone episodes reported yearly (FAS)

Visit	Number (%) of patients reporting at least one episode		Number of episodes reported	
	Untreated (N=69)	Nitisinone (N=69)	Untreated (N=69)	Nitisinone (N=69)
Baseline	18 (26.1)	22 (31.9)	29	48
New episodes since baseline				
Month 12	2 (2.9)	8 (11.6)	3	13
Month 24	6 (8.7)	10 (14.5)	10	17
Month 36	9 (13.0)	16 (23.2)	17	30
Month 48	11 (15.9)	16 (23.2)	22	34

Percentage calculated on N. Missing episodes are counted as no episodes.
Source: Table 10.2.2.1 – 8

There were no differences in renal stones episodes prevalence at 48 months between the two groups (p=0.719).

Table 27. Osteopenia of the hip (T-scores) at baseline and Month 48 (FAS)

Visit	Statistic	Untreated (N=69)		Nitisinone (N=69)	
		Value	Change from baseline	Value	Change from baseline
Baseline	N	64		68	
	Mean (SD)	-1.26 (0.98)		-1.30 (1.20)	
	Median (min; max)	-1.25 (-3.9; 1.3)		-1.30 (-3.5; 2.8)	
Month 48	Adjusted mean		-0.19		-0.05
	95% CI		-0.29; -0.09		-0.15; 0.05
	Adjusted mean (difference nitisinone-untreated)				0.14
	95% CI				0.00; 0.28
	p-value				0.045

n: Number of patients observed
Source: Table 10.2.2.1 – 9, Table 10.2.2.1 – 10

In nitisinone group more patients started bisphosphonates compared to untreated: n=9 (13%) in nitisinone; n=5 (7.2%) in untreated.

Table 28. Cumulative number of fractures reported yearly (FAS)

Visit	Number (%) of patients reporting at least one episode		Number of episodes reported	
	Untreated (N=69)	Nitisinone ((N=69)	Untreated (N=69)	Nitisinone (N
Baseline	11 (15.9)	15 (21.7)	21	21
New episodes since baseline				
Month 12	2 (2.9)	1 (1.4)	2	1
Month 24	2 (2.9)	1 (1.4)	2	1
Month 36	3 (4.3)	1 (1.4)	3	1
Month 48	7 (10.1)	3 (4.3)	8	3

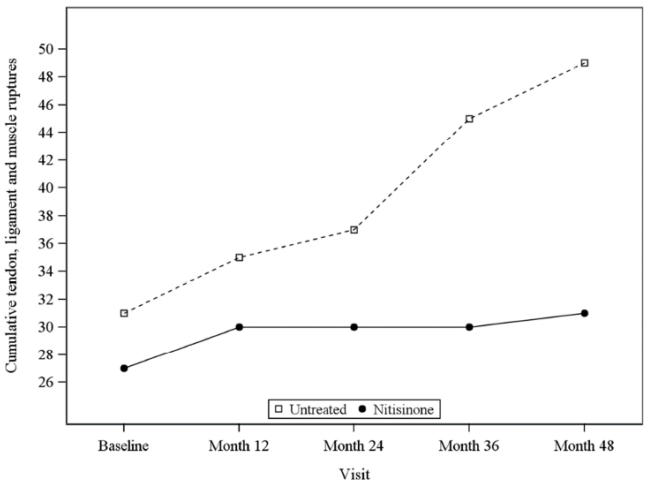
Percentage calculated on N. Missing episodes are counted as no episodes.
Source: Table 10.2.2.1 – 14

The difference between the treatment groups in change from baseline to Month 48 in number of fractures was not statistically significant (p = 0.160).

Table 29. Cumulative number of tendon, ligament and muscle ruptures reported yearly (FAS)

Visit	Number (%) of patients reporting at least one episode		Number of episodes reported	
	Untreated (N=69)	Nitisinone (N=69)	Untreated (N=69)	Nitisinone (N=69)
Baseline	21 (30.4)	17 (24.6)	31	27
New episodes since baseline				
Month 12	4 (5.8)	3 (4.3)	4	3
Month 24	4 (5.8)	3 (4.3)	6	3
Month 36	10 (14.5)	3 (4.3)	14	3
Month 48	13 (18.8)	4 (5.8)	18	4

Percentage calculated on N. Missing episodes are counted as no episodes.
Source: Table 10.2.2.1 - 16



Source: Figure 10.2.2.1 -7

Figure 12. Cumulative number of tendon, ligament and muscle ruptures over time (FAS)

Table 30. Number of patients with aortic sclerosis or stenosis at baseline and Month 48 (FAS)

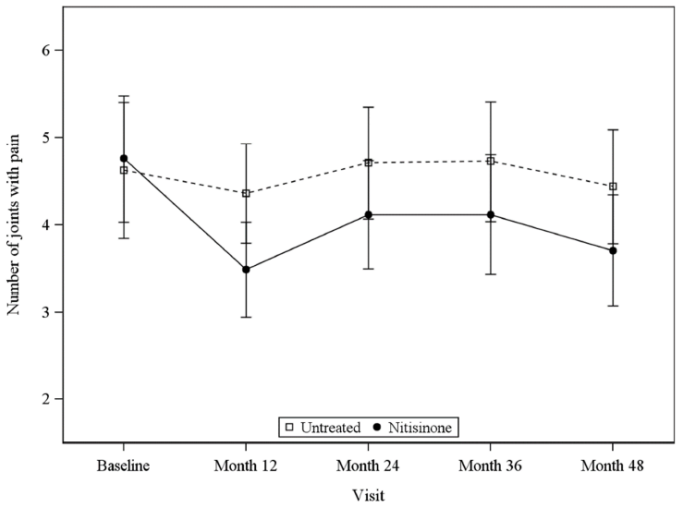
Visit	Sclerosis/Stenosis [a]	Untreated (N=69) n (%)	Nitisinone (N=69) n (%)
Baseline	n	69 (100.0)	69 (100.0)
	Normal	51 (73.9)	44 (63.8)
	Pts. with sclerosis or stenosis	18 (26.1)	25 (36.2)
	Sclerosis	10 (14.5)	15 (21.7)
	Mild stenosis	4 (5.8)	4 (5.8)
	Moderate stenosis	3 (4.3)	3 (4.3)
	Severe stenosis	1 (1.4)	3 (4.3)
	Missing	0 (0.0)	0 (0.0)
Month 48	Improved by 2 categories	0 (0.0)	0 (0.0)
	Improved by 1 category	19 (27.5)	16 (23.2)
	Unchanged	33 (47.8)	35 (50.7)
	Worsened by 1 category	1 (1.4)	4 (5.8)
	Worsened by 2 categories	0 (0.0)	0 (0.0)
	Missing	16 (23.2)	14 (20.3)
	p-value*		0.493

[a] Aortic sclerosis/stenosis is categorized as 'Normal', 'Sclerosis', 'Mild stenosis', 'Moderate stenosis' and 'Severe stenosis'
n: Number of patients observed
Percentage calculated on N (patients in treatment groups)
Source: Table 10.2.2.1 – 25, Table 10.2.2.1 – 26

Table 31. Number of joints with pain at baseline and Month 48 (FAS)

Visit	Statistic	Untreated (N=69) Value	Change from baseline	Nitisinone95% CI (N=69) Value	Change from baseline
Baseline	n	69		69	
	Mean (SD)	4.6 (3.3)		4.8 (3.0)	
	Median (min; max)	4.0 (0; 13))		4.0 (0; 12))	
Month 48	Adjusted mean		-0.2		-1.0
	95% CI		-0.9; 0.4		-1.6; -0.3
	Adjusted mean (difference nitisinone-untreated)				-0.7
	95% CI				-1.6; 0.1
	p-value*				0.103

n: Number of patients observed
Source: Table 10.2.2.1 – 31, Table 10.2.2.1 - 32



Source: Figure 10.2.2.1 -15

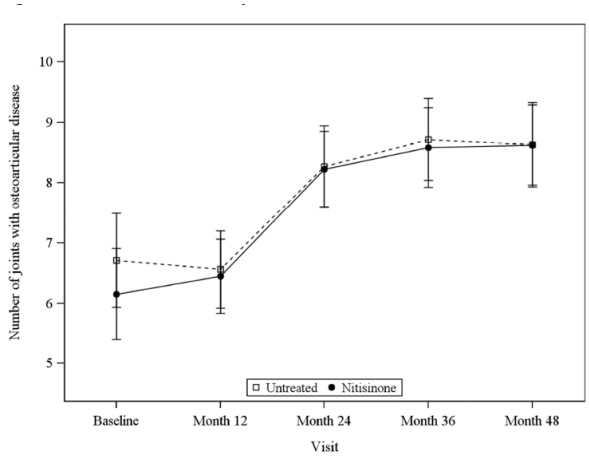
Figure 13. Number of joints with pain over time (FAS)

Table 32. Number of spinal regions with pain at baseline and Month 48 (FAS)

		Untreated (N=69)		Nitisinone (N=69)	
Visit	**Statistic**	**Value**	**Change from baseline**	**Value**	**Change from baseline**
Baseline	n	69		69	
	Mean (SD)	2.3 (1.2)		2.3 (1.3)	
	Median (min; max)	2.0 (0; 4)		2.0 (0; 4)	
Month 48	Adjusted mean		-0.2		-0.6
	95% CI		-0.5; 0.2		-0.9; -0.3
	Adjusted mean (difference nitisinone-untreated)				-0.5
	95% CI				-0.9; 0.0
	p-value*				0.048

n: Number of patients observed
Source: Table 10.2.2.1 – 33, Table 10.2.2.1 - 34

In the nitisinone group twice as many patients started new medications after randomization, regarding analgesic drugs, acetic acid derivatives and related substances, compared to placebo: n=19 (27.5%) in nitisinone; n=10 (14.5%) in untreated. A similar trend, but less important, was observed for analgesic drug in the class of propionic acid derivatives: n= 17 (24.6%) in nitisinone; n=12 (17.4%) in untreated.



Source: Figure 10.2.2.1 -17

Figure 14. Number of joints with ostoarticular disease over time (FAS)

Table 33. Number of patinets with scoliosis at baseline and Month 48

Visit	Scoliosis (Cobb angle)	Untreated (N=69) n (%)	Nitisinone (N=69) n (%)
Baseline	n	69 (100.0)	69 (100.0)
	<5	2 (2.9)	4 (5.8)
	5-20	65 (94.2)	63 (91.3)
	21-30	1 (1.4)	1 (1.4)
	>30	1 (1.4)	1 (1.4)
	Missing	0 (0.0)	0 (0.0)
Month 48	Improved by 2 categories	0 (0.0)	0 (0.0)
	Improved by 1 category	1 (1.4)	0 (0.0)
	Unchanged	49 (71.0)	51 (73.9)
	Worsened by 1 category	2 (2.9)	4 (5.8)
	Worsened by 2 categories	1 (1.4)	0 (0.0)
	Missing	16 (23.2)	14 (20.3)
	p-value*		0.594

[a] Scoliosis is categorized as '<5', '5-20', '21-30' and '>30'.
n: Number of patients observed.
Percentage calculated on N (patients in treatment groups)
Source: Table 10.2.2.1 – 45, Table 10.2.2.1 - 46

Table 34. Cumulative number of joint replacements reported yearly (FAS)

Visit	Number (%) of patients		Number of episodes	
	Untreated (N=69)	Nitisinone (N=69)	Untreated (N=69)	Nitisinone (N=69)
Baseline	12 (17.4)	17 (24.6)	26	35
New episodes since baseline				
Month 12	3 (4.3)	4 (5.8)	3	4
Month 24	6 (8.7)	8 (11.6)	7	9
Month 36	7 (10.1)	13 (18.8)	11	17
Month 48	10 (14.5)	17 (24.6)	16	24

Percentage calculated on N.
Missing episodes is counted as no episodes.
Created using akussi1.sas, akussi2.sas, akussi3.sas and rand_fas.sas version 1 in S:\Statistical Documents\AKU\Trials\SONIA 2\Final analysis\Final programming\Data by MG on 12DEC19:09:53:20.

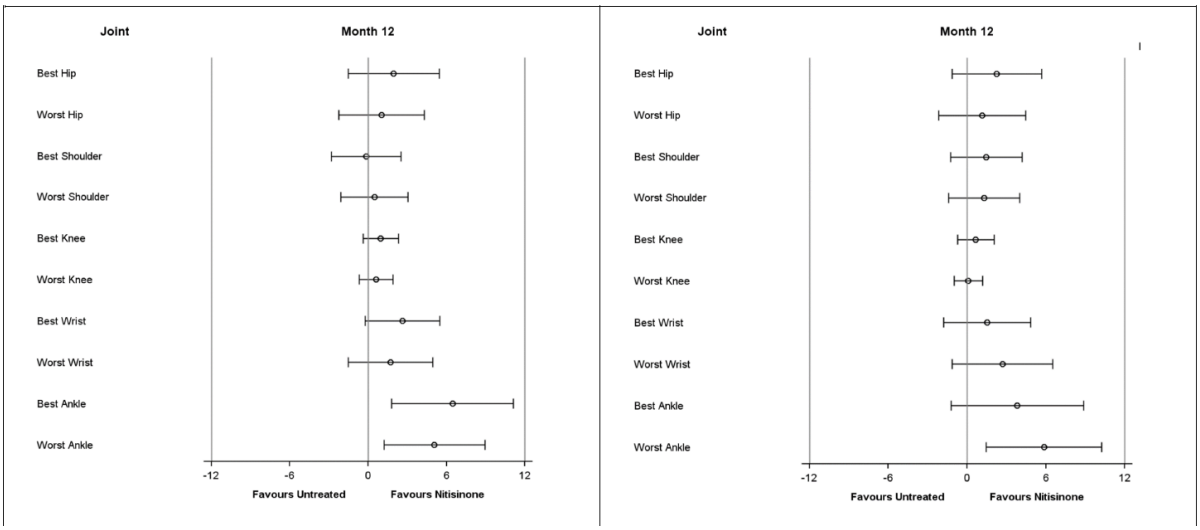
At baseline, a total of 26 patients in the study (18.8%) reported to have undergone at least 1 arthroscopy. At Month 48, no notable difference between the treatment groups in change from baseline was observed (p = 1.000).

At baseline, a total of 29 patients (21%) in had undergone at least 1 joint replacement. At Month 48, no notable difference between the treatment groups in change from baseline was observed (p = 0.881).

Change from baseline in range of joint motions are presented in Figure 15.

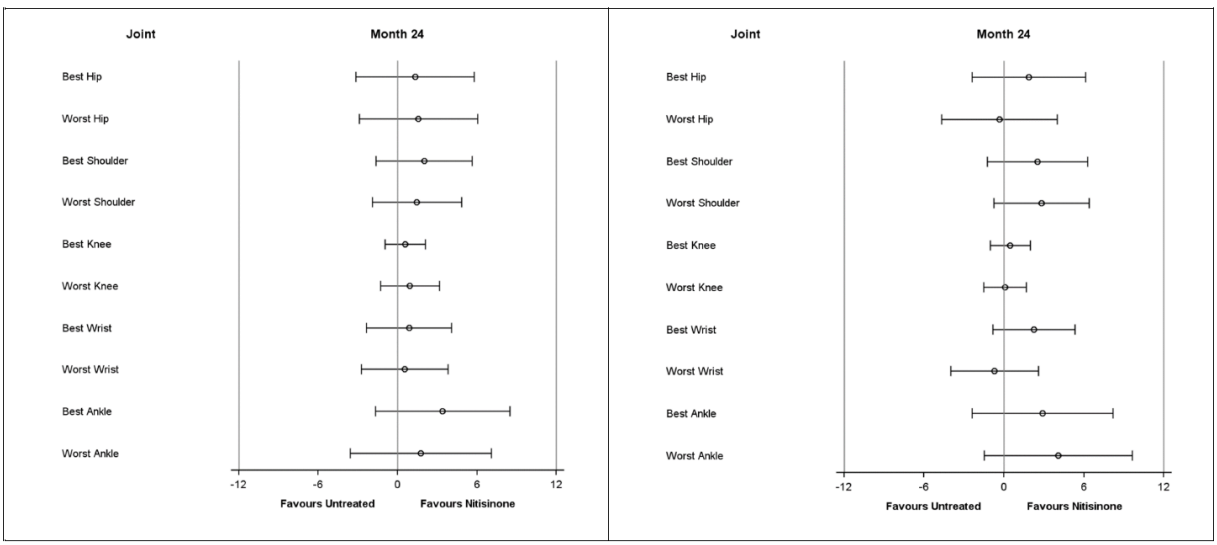
Active range of motion **Passive range of motion**

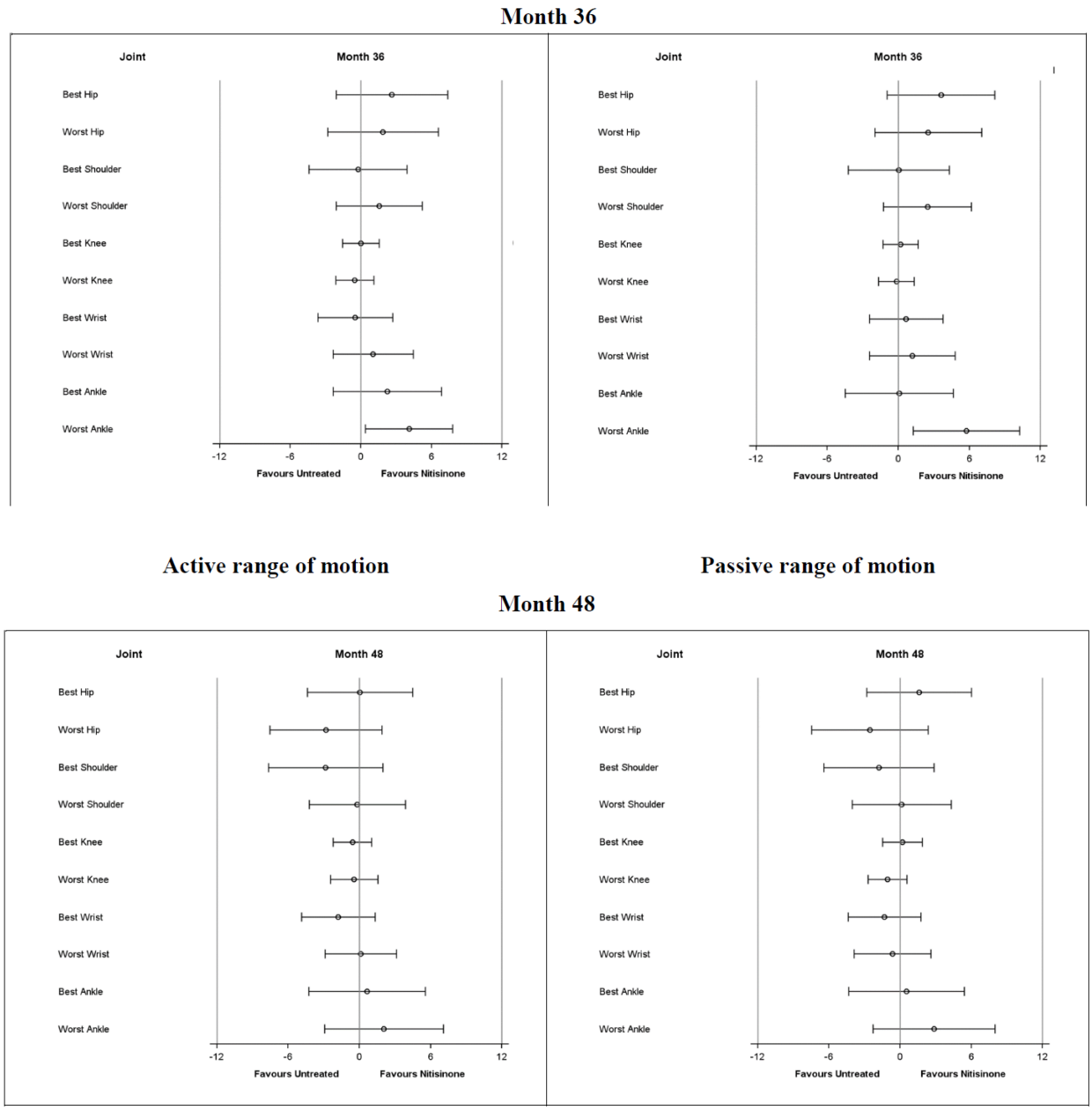
Month 12



Active range of motion **Passive range of motion**

Month 24

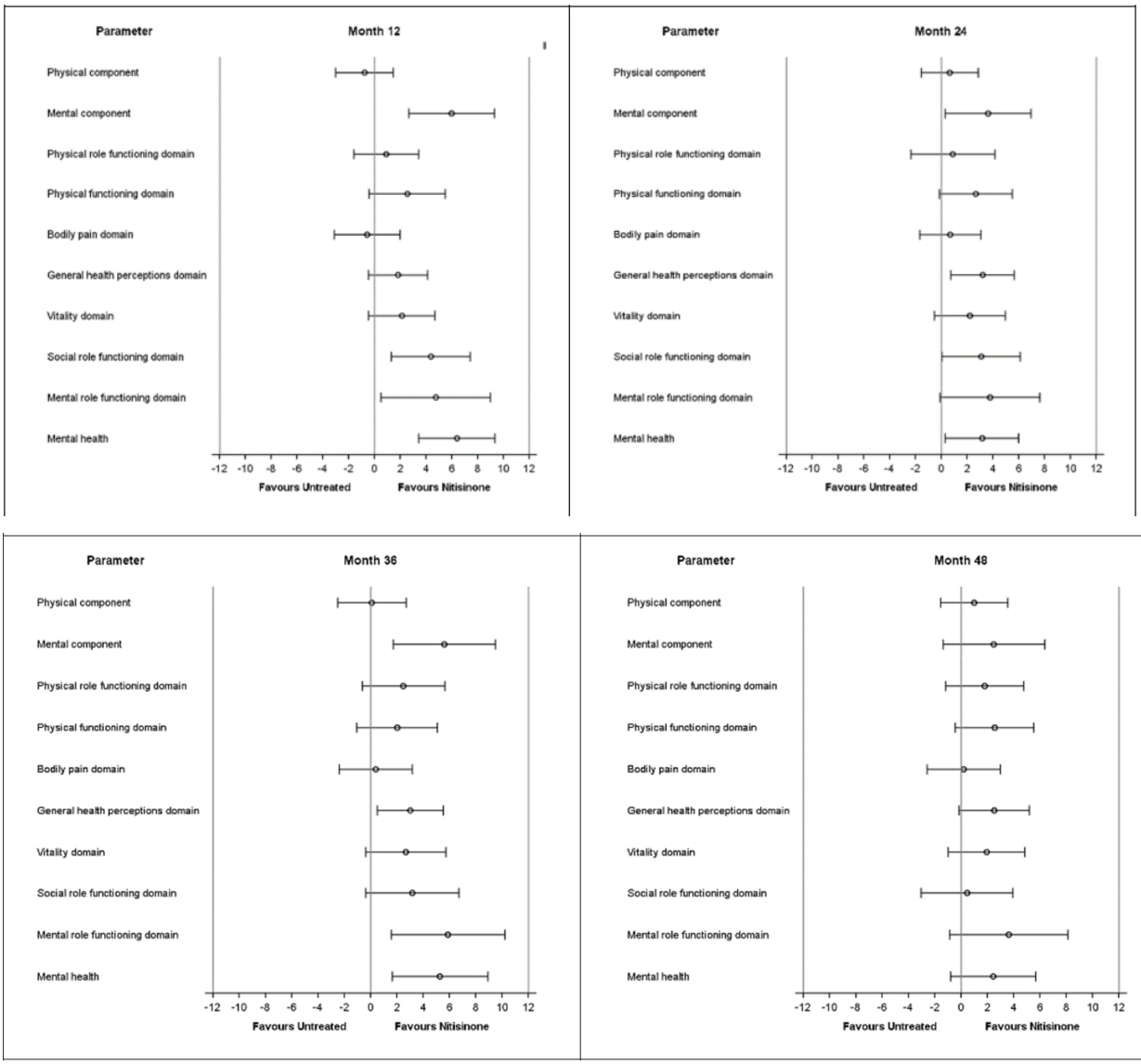




Graphs show adjusted mean (95% CI) change from baseline between nitisinone treated and untreated patients
Change from baseline is analyzed using a mixed model with repeated measurements (MMRM).
Source: Figure 10.2.3 – 1, Figure 10.2.3 – 2, Figure 10.2.3 – 3, Figure 10.2.3 – 4, Figure 10.2.3 – 6, Figure 10.2.3 – 6, Figure 10.2.3 – 7, Figure 10.2.3 – 8

Figure 15. Forest plots of the change from baseline in active (left) and passive (right) range of motion results yearly from Month 12 to month 48 (% of maximal normal value), FAS

Change from baseline in SF-36 Health Survey results yearly from Month 12 to Month 48 and results from the final item (excluded from the SF-36 Health Survey), self-evaluated transition item ("Compared to one year ago, how would you rate your health in general now?) are shown in Figure 16 and Table 35. respectively



Graphs show adjusted mean (95% CI) change from baseline between nitisinone treated and untreated patients
*Change from baseline is analyzed using a mixed model with repeated measurements (MMRM).
Source: Figure 10.2.4.1 – 1, Figure 10.2.4.1 – 2, Figure 10.2.4.1 – 3, Figure 10.2.4.1 – 4

Figure 16. Forest plots of the change from baseline in SF-36 Health Survey results yearly from Month 12 to Month 48 (FAS)

Table 35. Change from baseline in SF-36, self evaluated transition (SET) item (FAS)

Visit	Statistic	Untreated (N=69)	Nitisinone (N=69)
Month 12	Adjusted mean	-0.2	-0.6
	95% CI	-0.4; 0.0	-0.8; -0.4
	Adjusted mean (difference nitisinone-untreated)		-0.4
	95% CI		-0.7; -0.1
	p-value*		0.014
Month 24	Adjusted mean	0.2	-0.2
	95% CI	0.0; 0.4	-0.4; -0.1
	Adjusted mean (difference nitisinone-untreated)		-0.4
	95% CI		-0.7; -0.2
	p-value*		0.002
Month 36	Adjusted mean	0.1	-0.3
	95% CI	-0.2; 0.3	-0.5; -0.1
	Adjusted mean (difference nitisinone-untreated)		-0.3
	95% CI		-0.6; -0.1
	p-value*		0.021
Month 48	Adjusted mean	0.2	-0.4
	95% CI	-0.1; 0.4	-0.6; -0.2
	Adjusted mean (difference nitisinone-untreated)		-0.6
	95% CI		-0.9; -0.2
	p-value*		0.001

*Change from baseline is analyzed using a mixed model with repeated measurements (MMRM).
Higher score indicates worse quality of life.
Created using sf36.sas and _patientinfo.sas version 1 in S:\Statistical Documents\AKU\Trials\SONIA 2\Final analysis\Final programming\Data by MG on 12DEC19:10:22:48.

Ancillary analyses

During the procedure, further data were submitted to better understand the differences in baseline characteristics of the two comparison groups and support the consistency of the treatment effect observed in the main study SONIA-2.

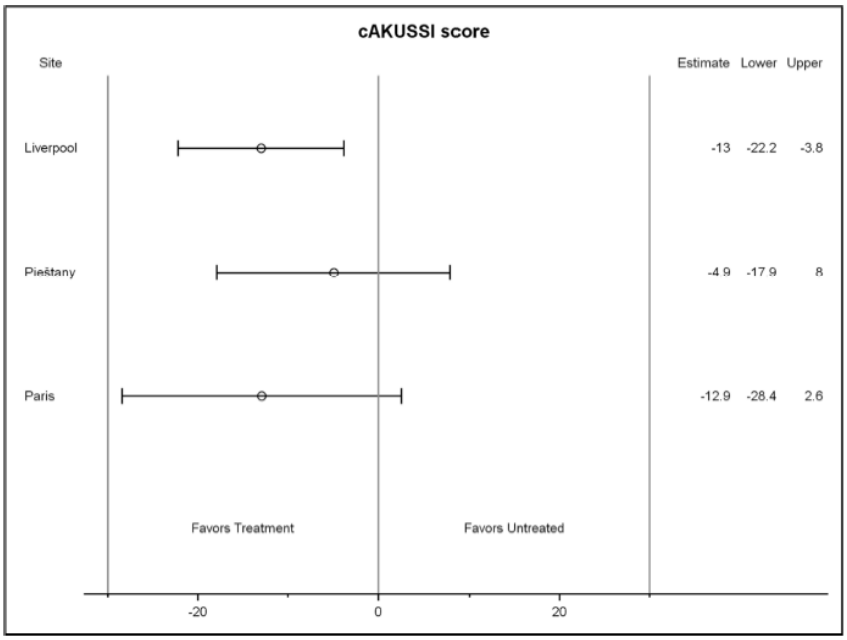
The age distribution and cAKUSSI score at Month 48 by sites were presented in Table 36 and Figure 17.

Table 36. Age distribution by site (FAS)

	Liverpool		Piešťany		Paris	
	Untreated (N = 21)	Nitisinone (N = 20)	Untreated (N = 32)	Nitisinone (N = 33)	Untreated (N = 16)	Nitisinone (N = 16)
Age (years)						
n	21	20	32	33	16	16
Mean (SD)	50.1 (10.25)	49.9 (11.59)	45.9 (10.55)	46.6 (12.53)	47.7 (8.75)	52.8 (6.30)
Median (Range)	48.0 (29 - 67)	52.0 (29 - 67)	46.5 (28 - 63)	46.0 (26 - 70)	49.5 (27 - 59)	53.5 (42 - 63)

Source: DEM_SITE_FAS_T.SAS 2020-06-24T07:10:42 Z9FRBE

The following figure shows a forest plot for cAKUSSI score by sites.



Source: CAKUS_SITE_FORREST1_FAS_F.SAS 2020-06-24T07:10:44 Z9FRBE

Figure 17 Forest plot: cAKUSSI effect at Month 48 by site (FAS)

Tabulated data with the baseline mean/median scores of the different subdomains of AKUSSI in the overall groups, by sex and age were also submitted. See Tables 37-39.

Table 37. Summary of changes from baseline in mAKUSSI items scores (FAS)

	Adjusted mean (95% CI) difference nitisinone vs. untreated.	Favors nitisinone?
Prostate stones[a]	-0.2 (-1.8, 1.3)	Yes
Renal stones	0.5 (-1.1, 2.2)	No
Osteopenia of the hip	-0.3 (-0.6, 0.1)	Yes
Fractures	-0.7 (-1.3, -0.1)	Yes
Ruptures	-1.8 (-3.2, -0.4)	Yes
Aortic valve sclerosis/ stenosis	-0.3 (-0.9, 0.4)	Yes
Hearing impairment	0.0 (-0.4, 0.5)	No
Clinical joint pain	-0.7 (-1.6, 0.1)	Yes
Non-spine osteoarticular disease	-0.2 (-2.6, 2.2)	Yes
Arthroscopies	-0.1 (-0.3, 0.1)	Yes
Joint replacements	0.5 (-0.5, 1.5)	No
Clinical spinal pain	-0.9 (-1.8, 0.0)	Yes
Osteoarticular disease of the spine	-0.3 (-1.8, 1.1)	Yes
Kyphosis	0.1 (-0.3, 0.4)	No
Scoliosis	0.0 (-0.2, 0.2)	No
Total 'Yes'		10/15

Source: Table A1

Table 38. Summary of changes from baseline in mAKUSSI items scores by age (FAS) Adjusted mean (95%CI) difference nitisinone vs untreated

	≤ 55 years	Favors nitisinone?	> 55 years	Favors nitisinone?
Prostate stone	-0.4 (-1.9, 1.2)	Yes	-0.2 (-2.9, 2.4)	Yes
Renal stones	0.7 (-1.5, 2.9)	No	0.1 (-1.4, 1.6)	No
Osteopenia of the hip	-0.1 (-0.5, 0.3)	Yes	-0.4 (-1.3, 0.5)	Yes
Fractures	0.0 (-0.8, 0.7)	No	-2.5 (-3.4, -1.5)	Yes
Ruptures	-1.7 (-3.5, 0.1)	Yes	-1.9 (-3.9, 0.2)	Yes
Aortic valve sclerosis/ stenosis	-0.4 (-1.2, 0.3)	Yes	0.1 (-1.5, 1.7)	No
Hearing impairment	0.1 (-0.4, 0.6)	No	-0.3 (-1.3, 0.7)	Yes
Clinical joint pain	-0.4 (-1.3, 0.5)	Yes	-1.6 (-3.7, 0.6)	Yes
Non-spine osteoarticular disease	-1.3 (-4.3, 1.7)	Yes	1.3 (-1.8, 4.3)	No
Arthroscopies	0.0 (-0.2, 0.2)	No	-0.3 (-0.7, 0.1)	Yes
Joint replacements	0.6 (-0.3, 1.6)	No	-0.2 (-2.5, 2.2)	Yes
Clinical spinal pain	-0.5 (-1.6, 0.6)	Yes	-1.9 (-3.6, -0.3)	Yes

Osteoarticular disease of the spine	-0.3 (-2.1, 1.5)	Yes	-0.2 (-2.8, 2.5)	Yes
Kyphosis	0.2 (-0.2, 0.5)	No	-0.3 (-1.2, 0.5)	Yes
Scoliosis	0.0 (-0.1, 0.2)	No	-0.2 (-0.7, 0.2)	Yes
Total 'Yes'		8/15		12/15

Source: Table A2

Table 39. Summary of changes from baseline in mAKUSSI items scores by sex (FAS) Adjusted mean (95%CI) difference nitisinone vs untreated

	Male	Favors nitisinone?	Female	Favors nitisinone?
Prostate stone	-0.2 (-1.8, 1.3)	Yes	NA	NA
Renal stones	1.0 (-1.5, 3.5)	No	-0.4 (-1.2, 0.4)	Yes
Osteopenia of the hip	-0.2 (-0.7, 0.3)	Yes	-0.3 (-1.0, 0.4)	Yes
Fractures	-0.3 (-1.0, 0.4)	Yes	-1.3 (-2.5, -0.2)	Yes
Ruptures	-2.6 (-4.7, -0.5)	Yes	-0.6 (-1.7, 0.5)	Yes
Aortic valve sclerosis/ stenosis	-0.4 (-1.2, 0.5)	Yes	-0.2 (-1.4, 1.0)	Yes
Hearing impairment	0.1 (-0.5, 0.8)	No	-0.2 (-0.9, 0.5)	Yes
Clinical joint pain	-1.3 (-2.2, -0.3)	Yes	0.7 (-1.0, 2.4)	No
Non-spine osteoarticular disease	0.0 (-3.1, 3.1)	No	-0.6 (-3.7, 2.5)	Yes
Arthroscopies	-0.3 (-0.5, 0.0)	Yes	0.2 (-0.1, 0.5)	No
Joint replacements	0.8 (-0.4, 2.1)	No	-0.1 (-1.8, 1.6)	Yes
Clinical spinal pain	-1.4 (-2.5, -0.3)	Yes	0.0 (-1.6, 1.7)	No
Osteoarticular disease of the spine	-0.6 (-2.3, 1.1)	Yes	-0.1 (-3.0, 2.8)	Yes
Kyphosis	-0.1 (-0.5, 0.2)	Yes	0.4 (-0.3, 1.1)	No
Scoliosis	0.0 (-0.1, 0.2)	No	0.2 (-0.4, 0.7)	No
Total 'Yes'		10/15		9/14

Source: Source: Table A3
NA = Not applicable

Additional efficacy analyses were also performed based on patients U-HGA levels (<300 umol/L and >300 umol/L) and are presented in Tables 40 and 41. The age distribution is presented in Table 42.

Table 40. Change from baseline in cAKUSSI scores by u-HGA24 classified by the Month 48 uHGA24 value[a] (FAS, nitisinone only)

	< 300 µmol Nitisinone (N = 41)	≥ 300 µmol Nitisinone (N = 14)
Baseline		
n	41	14
Mean (SD)	93.3 (33.9)	67.6 (30.5)
Median (Range)	95.0 (16 - 152)	61.0 (30 - 127)
Month 48		
Adjusted mean (95% CI)	9.0 (4.2, 13.8)	7.4 (-1.0, 15.8)
Adjusted mean (95% CI) difference vs. <300 µmol		-1.6 (-11.1, 7.9)
p-value		0.7314

Source: CAKUS3_UHGA4_FAS_T.SAS 2020-06-24T07:10:34 Z9FRBE
[a] Patients without a month 48 value are excluded.
NOTE: Changes from baseline in the total cAKUSSI scores is analyzed using a mixed model with repeated measurements (MMRM) and an underlying normal distribution. If an item is missing at baseline, then that item is excluded from the calculation of cAKUSSI for that patient at all following visits. If an item is missing for a postbaseline visit, but available at least at baseline, then the last available observation is carried forward (LOCF).

Table 41. Change from baseline in mAKUSSI scores by u-HGA24 classified by the Month 48 uHGA24 value[a] (FAS, nitisinone only)

	< 300 µmol Nitisinone (N = 41)	≥ 300 µmol Nitisinone (N = 14)
Baseline		
n	41	14
Mean (SD)	62.4 (28.7)	40.8 (21.4)
Median (Range)	61.0 (4 - 117)	42.5 (10 - 77)
Month 48		
Adjusted mean (95% CI)	11.1 (7.0, 15.2)	7.4 (0.2, 14.5)
Adjusted mean (95% CI) difference vs. <300 µmol		-3.7 (-11.9, 4.5)
p-value		0.3663

Source: MAKUS3_UHGA4_FAS_T.SAS 2020-06-24T07:10:37 Z9FRBE
[a] Patients without a month 48 value are excluded.
NOTE: Changes from baseline in the total mAKUSSI scores is analyzed using a mixed model with repeated measurements (MMRM) and an underlying normal distribution. If an item is missing at baseline, then that item is excluded from the calculation of mAKUSSI for that patient at all following visits. If an item is missing for a postbaseline visit, but available at least at baseline, then the last available observation is carried forward (LOCF).

Table 42. Age by u- HGA24 classified by the Month 48 uHGA24 value[a] (FAS)

	< 300 umol		≥ 300 umol	
	Untreated (N = 0)	Nitisinone (N = 42)	Untreated (N = 54)	Nitisinone (N = 14)
Age (years)				
n		42	54	14
Mean (SD)		50.7 (9.90)	47.3 (9.48)	40.6 (11.67)
Median (Range)		53.5 (27 - 66)	48.5 (28 - 63)	39.0 (26 - 66)

Source: DEM3_UHGA_FAS_T.SAS 2020-06-24T07:10:39 Z9FRBE
a Patients without a month 48 value are excluded.

There were only 14 patients in the group with u-HGA24 ≥ 300 µmol and 41 patients with u-HGA24 < 300 µmol, when they were grouped based on u-HGA24 at Month 48.

Patients with u-HGA24 values <300 µmol had cAKUSSI and mAKUSSI scores at baseline that were approximately 38% and 57% higher than patients with u-HGA24 values ≥300 µmol. Furthermore, the group with u-HGA24 ≥300 µmol was about 10 years younger on average than the group with u-HGA24 <300 µmol.

An alternative analysis, based on each patient's most common u-HGA24 category, is presented in the following Tables 43 and Table 44. Age distribution is presented in Table 45.

Table 43. Change from baseline in cAKUSSI scores by u-HGA24 classified according to most common category[a] (FAS, nitisinone only)

	< 300 µmol Nitisinone (N = 58)	≥ 300 µmol Nitisinone (N = 11)
Baseline		
n	58	11
Mean (SD)	92.8 (33.5)	57.1 (21.7)
Median (Range)	97.5 (16 - 152)	51.0 (34 - 97)
Month 48		
Adjusted mean (95% CI)	6.6 (0.6, 12.6)	5.0 (-8.6, 18.6)
Adjusted mean (95% CI) difference vs. <300 µmol		-1.6 (-16.5, 13.3)
p-value		0.8316

Source: CAKUS_UHGA4_FAS_T.SAS 2020-06-24T07:10:26 Z9FRBE
[a] If a patient has an equal number of visits in each category, the category related to the last visit is used.
NOTE: Changes from baseline in the total cAKUSSI scores is analyzed using a mixed model with repeated measurements (MMRM) and an underlying normal distribution. If an item is missing at baseline, then that item is excluded from the calculation of cAKUSSI for that patient at all following visits. If an item is missing for a postbaseline visit, but available at least at baseline, then the last available observation is carried forward (LOCF).

Table 44. Change from baseline in mAKUSSI scores by u-HGA24 classified according to most common category[a] (FAS, nitisinone only)

	< 300 µmol Nitisinone (N = 58)	≥ 300 µmol Nitisinone (N = 11)
Baseline		
n	58	11
Mean (SD)	60.8 (26.6)	35.8 (18.1)
Median (Range)	60.5 (4 - 117)	34.0 (10 - 65)
Month 48		
Adjusted mean (95% CI)	9.0 (4.6, 13.4)	5.7 (-4.3, 15.7)
Adjusted mean (95% CI) difference vs. <300 µmol		-3.3 (-14.2, 7.6)
p-value		0.5477

MAKUS_UHGA4_FAS_T.SAS 2020-06-24T07:10:28 Z9FRBE
[a] If a patient has an equal number of visits in each category, the category related to the last visit is used.
NOTE: Changes from baseline in the total mAKUSSI scores is analyzed using a mixed model with repeated measurements (MMRM) and an underlying normal distribution. If an item is missing at baseline, then that item is excluded from the calculation of mAKUSSI for that patient at all following visits. If an item is missing for a postbaseline visit, but available at least at baseline, then the last available observation is carried forward (LOCF).

Table 45. Age by u-HGA24 classified according to most common category[a] (FAS, nitisinone only)

	< 300 µmol Nitisinone (N = 58)	≥ 300 µmol Nitisinone (N = 11)
Age (years)		
n	58	11
Mean (SD)	50.5 (10.83)	40.8 (10.25)
Median (Range)	53.0 (26 - 70)	41.0 (28 - 59)

Source: DEM_UHGA_FAS_T.SAS 2020-06-24T07:10:31 Z9FRBE
[a] If a patient has an equal number of visits in each category, the category related to the last visit is used.

Proportion of patients (%) achieving response based on different response criteria were submitted to support the consistency of the treatment effects observed in SONIA 2. See Table 46.

Table 46. Proportion (%) of patients achieving response based on different response criteria (FAS, patients completing Month 48)

	Number (%) of patients			
Responder defined as (change from baseline)	Nitisinone < 300 µmol (N = 41)	Nitisinone ≥ 300 µmol (N = 14)	Total nitisinone (N = 55)	Untreated (N = 53)
mAKUSSI increase by max 4 points together with a reduction of at least 4 points in eye pigmentation	2 (4.9)	0	2 (3.6)	2 (3.8)
mAKUSSI increase by max 4 points together with no increase in eye pigmentation	15 (36.6)	5 (35.7)	20 (36.4)	12 (22.6)
No increase in mAKUSSI together with no increase in eye pigmentation	10 (24.4)	5 (35.7)	15 (27.3)	8 (15.1)
mAKUSSI increase by max 4 points	15 (36.6)	7 (50.0)	22 (40.0)	19 (35.8)
No increase in mAKUSSI	10 (24.4)	6 (42.9)	16 (29.1)	12 (22.6)
A reduction of at least 4 points in eye pigmentation	4 (9.8)	3 (21.4)	7 (12.7)	3 (5.7)
No increase in eye pigmentation	37 (90.2)	11 (78.6)	48 (87.3)	32 (60.4)

Source: MERESP_UHGA_FAS_T.SAS 2020-06-24T07:10:04 Z9FRBE

Summary of main study(ies)

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Title: An international, multicenter, randomized, evaluator-blinded, no-treatment controlled, parallel-group study to assess the efficacy and safety of once daily nitisinone in patients with alkaptonuria after 12 months of treatment, followed by an additional 36-month treatment period			
Study identifier	SONIA-2, EudraCT number: 2013-001633-41.		
Design	randomized, open-label, evaluator-blinded, parallel-group study with an untreated control group. Patients were randomized to receive either nitisinone or no treatment (control).		
	Duration of main phase::		48 months
Hypothesis	Superiority		
Treatments groups	Nitisinone		nitisinone 10 mg/day
	No-Treatment		no treatment
Endpoints and definitions	Primary endpoint	**u-HGA-24h**	Urinary HGA 24h (umol/L) in patients with AKU after 12 months
	Secondary endpoint	**u-HGA-24h**	Urinary HGA 24h (umol/L) in patients with AKU after 48 months
	Secondary endpoint	**s-HGA**	Serum HGA (umol/L) after 12 months
	Secondary endpoint	**cAKUSSI**	cAKUSSI score at month 48, change from baseline
	Secondary endpoint	**mAKUSSI**	mAKUSSI score at month 48, change from baseline
Database lock	13 May 2019		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and timepoint description	Intent to treat, 12 months and 48 months		
Descriptive statistics and estimate variability	Treatment group	No-treatment	Nitisinone
	Number of subjects	69	69
	u-HGA-24h 12 months (Adjusted geometric mean)	26027.9	85.7
	95% CI	21649.6; 31291.8	71.8; 102.2

	u-HGA-24h 48 months (Adjusted geometric mean)	29936.1	158.1
	95% CI	22090.6; 40568.0	117.0; 213.6
	cAKUSSI 48 months (Adjusted mean)	16.1	7.4
	95% CI	10.7; 21.4	2.1; 12.8
	mAKUSSI 48 months (Adjusted mean)	12.4	8.8
	95% CI	8.1; 16.7	4.5; 13.1

Effect estimates per comparison	**u-HGA-24h** 12 months	Comparison groups	Nitisinone vs no-treatment
		Adjusted geometric mean (ratio nitisinone/untreated)	0.003
		95% CI	0.003; 0.004
		P-value	<0.001
	u-HGA-24h 48 months	Comparison groups	Nitisinone vs no-treatment
		Adjusted geometric mean (ratio nitisinone/untreated)	0.005
		95% CI	0.003; 0.008
		P-value	<0.001
	s-HGA 12 months	Comparison groups	Nitisinone vs no-treatment
		Adjusted geometric mean (ratio nitisinone/untreated)	0.01
		95% CI	0.01; 0.02
		P-value	<0.001
	cAKUSSI 48 months	Comparison groups	Nitisinone vs no-treatment
		Adjusted mean (difference nitisinone-untreated)	-8.6
		95% CI	-16.0; -1.2
		P-value	0.023
	mAKUSSI	Comparison groups	Nitisinone vs no-treatment

	48 months	Adjusted mean (difference nitisinone-untreated)	-3.6
		95% CI	-9.6; 2.4
		P-value	0.234

2.4.3. Discussion on clinical efficacy

Design and conduct of clinical studies

In SONIA-1, the primary endpoint (u-HGA levels) was considered appropriate. The CHMP recognized u-HGA levels as an informative surrogate to evaluate treatment effects. According to the CHMP Scientific Advice (SA), increased levels of HGA are the leading cause of ochronosis in patients, that in turn results in the co-morbidities effects of alkaptonuria. Even if no human data are available, there are very strong evidence in mouse models: when mouse models are started on nitisinone very early, this completely prevents ochronosis development. In human patients with AKU who also develop renal failure, an accelerated ochronosis is seen because of the reduced ability to excrete HGA. From the CHMP viewpoint, all these observations support the validity of u-HGA as surrogate/pharmacodynamic endpoint to test treatment effects.

The design of the study seems appropriate. The number of subjects recruited for each arm is small (n=8) but it must be considered that the endpoints assessed are biochemical markers strictly related to the pharmacodynamic mechanism of the investigational product, and thus they are very sensitive indicators of the enzymatic inhibition by nitisinone.

The study did not use placebo as control because of the difficulty to perform an actual double-blind study since the treatment is associated with absence of change in urine colour, a phenomenon that can be easily recognized by both patients and investigators. However, since the endpoint was a biochemical marker (u-HGA) the lack of blinding of the study should not have impaired the reliability of the efficacy results.

From the clinical development program of Orfadin in hereditary tyrosinemia type 1 (HT-1), it was known that a dose of 2 mg/day could reduce urinary excretion of HGA by 95%, while increasing mean tyrosine levels to >700 µmol/L. The MAH originally planned to study doses ranging from 0.5 to 4 mg/day aiming at finding a dose that would significantly reduce HGA without giving high tyrosine levels with a potential of causing ocular adverse events. During the scientific advice, the CHMP expressed a wish to find a dose that would normalize HGA. This resulted in doses of 1, 2, 4 to 8 mg being used in the dose-response SONIA 1 study in patients with AKU. In SONIA-1, the selection of the dose range is not entirely understandable given that the currently approved strengths in the HT-1 indication include 10mg and 20 mg. However, results showed that the levels of u-HGA 24h markedly decreased in a clear dose-dependent manner with nitisinone treatment. In the 8 mg group (the highest dose used) the reduction (vs baseline) was about 99%. Furthermore, none of the treated patients reached the normal u-HGA levels observed in non-AKU subjects (normal values were indeed very low and quantifiable only in a fraction of normal subjects) and a ceiling effect was seen at serum concentration of nitisinone of about 3 umol/L (mostly reached with the 8 mg dosage). Despite the latter finding (ceiling effect at 8 mg dose), the CHMP noted that 10 mg dose was used for the pivotal study SONIA-2.

During the conduct of the study of SONIA-1, a few errors and mixed up samples and incorrect sample times were identified. Considering the low number of subjects in SONIA-1, the MAH provided further details on measures put in place to minimize errors in handling and analysis of biological samples, including standard measures to ensure protocol adherence in SONIA-1 and 2. These were considered sufficient to support the validity/reliability of studies.

In SONIA-2, the study design, including the open label nature, was discussed during CHMP scientific advice and considered acceptable given that the characteristic coloration of urines induced by nitisinone prevents blinding.

As stated by the MAH, the lack of a double-blind design is unlikely to have introduced bias in evaluation of the primary endpoint or those secondary endpoints which were based on serum analyses or evaluator-blinded assessments. However, reporting of subjective assessments potentially introduced bias for some of the secondary endpoints, such as pain, QoL assessments, for the efficacy aspects. From the CHMP viewpoint, even with an evaluator blinded assessment, patients could not unintentionally transfer any information about their treatment allocation to the evaluators and these limitations should also be considered.

Regarding patient selection criteria, it appears the genetic diagnosis was not required in order to diagnose AKU. All patients were already known to be affected by AKU. Ochronosis was not a specific criterion for enrolling the patients: all the Investigators were very familiar with AKU and thus able to assess the presence of sufficient signs of disease progression, according to the MAH. Literature data indicate that the amount of HGA excreted per day in individuals with alkaptonuria is usually between one and eight grams. Although not specified in the inclusion criteria, at baseline, the mean daily excretion of HGA was > 35000 µmol/day in both treatment groups, corresponding to approximately 5.88grams per day. The minimum amount excreted in any patient was 7457 µmol (1.25 grams) and the maximum 84320 µmol (14.2 grams). Therefore, the observed baseline uHGA levels are deemed appropriate to correctly identify the patient population with AKU. No information on the number of patients with chronic back / joint pain at baseline was provided; however, the high proportions of patients taking analgesic drugs at baseline is considered indicative of the presence of pain in the large majority of the recruited population. The CHMP noted that no radiographic confirmation of arthritis is listed in the inclusion criteria.

Among the inclusion criteria, age ≥25 years was required for the enrolment. Since the clinical manifestations of AKU usually start between 20-30 years of age, the age threshold was introduced in order to increase the sensitivity of the study. This justification seems reasonable. The CHMP noted that subjects > 70 years were not enrolled, this information was recommended for inclusion in the SmPC.

The exclusion criteria include patients with low blood cells count. This is appropriate since, in previous experience with nitisinone for treatment of patients affected by HT-1, AEs related to lower blood cells count were described.

The choice of a pharmacodynamic endpoint as the primary outcome variable (u-HGA24) was deemed acceptable by the CHMP as it directly relates to the mechanism of action of nitisinone and to what is understood as the pathogenic mechanism of the disease. However, it was noted that during the scientific advice, the CHMP recommended that the correlation/consistency between treatment effect on the primary endpoint and long-term clinical outcomes should be demonstrated in order to support nitisinone benefit in the claimed indication.

Upon the CHMP request that a link should be shown, at the end of the study, between the primary pharmacodynamics endpoint (u-HGA levels) and clinical outcomes, the evaluation of treatment effect on the All Alkaptonuria Severity Score Index (AKUSSI score) was included among the secondary endpoints.

The AKUSSI score measures disease severity in clinical, joint and spine domains. The limits of the AKUSSI scoring system are acknowledged: many items are subjective in nature; the number of points for each item does not necessary reflect the relative clinical importance or the underlying seriousness of the disease; the weight given to each severity score may not be optimal. However, in the absence of more sensitive tools in such a rare disorder, the AKUSSI score allows to assess, in a comprehensive manner, treatment effect on the complex clinical manifestations of the disease.

The study was powered to see a difference of 4 points on the AKUSSI score between control and treatment. The choice of 4 points as minimal clinically important difference is justified by the MAH by the fact that this would corresponded to a decrease in disease progression by 50%, assuming an increase of 2 points per year in untreated patients, which is what was found in the original AKUSSI study. Moreover, according to the MAH, as AKU is a slowly progressing disease it could be difficult to determine what level of reduction is considered clinically relevant and, as it is expected that the reduced progression will continue as long as the patient is treated with nitisinone, the observed effect after 4 years would be considerably more marked after a longer period.

The primary analysis was a MMRM with treatment, visit and treatment-by-visit interaction included as fixed factors and subject-within-site as a random factor. Adjusted geometric means and ratio of adjusted geometric means were used to express differences from baseline values, which is considered acceptable by the CHMP. Data from beyond the 12-month visit were not included in the primary analysis. Both intermittently and monotonously missing data were observed and imputed in two steps tipping point analysis.

No control for multiplicity was implemented and thus all secondary endpoints are descriptive in nature. This limits the interpretation of treatment effect on clinical outcomes. Given that the surrogacy of the primary pharmacodynamic endpoint for long-term clinical outcomes is not demonstrated, the lack of strong statistical support impact on the strength of the evidence.

Efficacy data and additional analyses

Some imbalance in patient's demographic and clinical characteristics were observed between the untreated (control) and treated groups. The proportion of patients > 55 years old (33.3% vs 27.5%) as well as of female patients (42% vs 34.8%) was higher in the nitisinone group compared to control. Patients in the nitisinone group showed overall a more compromised clinical status (around 6-10% difference is observed in many clinical characteristics between groups). In particular, although the percentage of subjects undergoing arthroscopies, that are indicative of overt joint damage, were similar, total joint failure requiring joint replacement was higher at baseline in the nitisinone group (24.6% vs 17.4%) compared to control. Small imbalances were also observed in the use of analgesic and anti-inflammatory medications more frequent in the nitisinone group. The higher disease burden at baseline in the nitisinone group could be attributed to the larger proportions of patients > 55 years. Patients <55 years were 96 (46 nitisinone + 50 control), whereas patients > 55 years were 42 (23 nitisinone + 19 control). Further data with the baseline mean/median scores of the different subdomains of AKUSSI in the overall groups, by sex and age confirmed the presence of imbalances in many clinical aspects at baseline, apparently suggestive of a more severe condition in the nitisinone group. Consistent with the literature reporting women score, on average, 6 units less in for the AKUSSI score in females than males of the same age (Cox and Ranganath 2011), higher baseline scores in male patients compared to females were observed in SONIA 2. Similar findings were observed in patients aged >55 years vs $\leq$55 years (mean score of 69.8 vs 48.2, respectively), in the untreated and treated groups. Baseline imbalances are also noted with the additional efficacy analysis based on each patient's most common u-HGA24 category.

Nitisinone treatment dramatically reduced the excretion of u-HGA24, reaching a nadir at month 3. Thereafter, u-HGA24 levels were maintained with minor fluctuations throughout the observation period of 4 months. At month 12, u-HGA24 levels (umol/L) were 85.7 (95 CI: 71.8-102.2) in the nitisinone group vs 26027.9 (95% CI: 21649.6-31291.8) in the untreated group. Whilst some observations could question the treatment effect e.g physiological levels of u-HGA24 were very low and undetectable in many subjects in the pivotal study, these levels were not reached in AKU patients treated with nitisinone group; the proportion of subjects who had u-HGA levels below the pre-defined cut-off of 300 umol was 88.6% at month 12, but decreased to 59.4% (mean u-HGA24: 158.1) at month 48, the clinical relevance of these findings remain uncertain, especially since the u-HGA24 targeted level was set arbitrarily. One plausible explanation to the reported reduction in the number of responders could be an effect due to patient compliance to treatment, that was indeed lower at month 48 (85.5%) compared to month 12 (100.0%).

Some patients who experienced keratopathy were switched to a lower dose of nitisinone, 2 mg, but u-HGA24 levels increased by 10-fold after the change of dose.

The cAKUSSI score showed a statistically significant result for nitisinone: difference nitisinone-control at month 48 was -8.6 (95% CI: -16.0, -1.2; p=0.023). However, results were clearly driven by treatment effect on eye pigmentation, and once the pigmentation variable is removed, as it is in the mAKUSSI, the difference between nitisinone and control is reduced to only -3.6 points (95% CI: -9.6, 2.4; p=0.234). Treatment effect on the eye pigmentation component of the cAKUSSI score, resulted in a difference from control of -2.5 points (95% CI: -3.9, -1.0; p=0.001), likely indicating a reduced rate of HGA precipitation in the eye. It is at present not known if a similar effect in other tissues is also observed.

The lack of a significant gain over the control group using mAKUSSI score did not allow to extrapolate that the reduced HGA precipitation in the eye may be considered a proxy for clinical benefit. However, it cannot be excluded that the follow up time of 4 years was too short to detect relevant changes in other disease manifestations. Moreover, the scoring system used in AKUSSI is suboptimal, as the same score is assigned to morbid variables that may require longer times to develop and are expression of different stages of disease severity. Higher mAKUSSI scores at baseline were observed in patients aged >55 years vs $\leq$55 years (mean score of 69.8 vs 48.2, respectively), and in the nitisinone group compared to control. When treatment effect was analysed by age and sex all subgroups reached or exceeded the pre-defined difference of 4 points between nitisinone and control, in both cAKUSSI and mAKUSSI scores, except for the mAKUSSI score in patients <55 years, suggesting heterogeneity in the untreated and treated groups may have contributed to the lack of effect in the total population. While acknowledging that the analysis was adjusted with respect to baseline, different severity of the baseline disease could have affected score sensitivity to detect improvement. At the CHMP request, the MAH further clarified that the primary analysis was also adjusted for age and site.

An imbalance in cAKUSSI score at baseline was observed in the Paris center with a score of 75.4 vs 100.1 in control and nitisinone groups, respectively, compared to patients in Liverpool (99.1 vs 96.9), and in Piešťany (70.8 vs 74.7). Albeit the numbers were low, the CHMP was concerned that in Piešťany, i.e. in the country with the larger prevalence of the disease, and where it is assumed that the clinical knowledge of the disease is greater, the numerical difference between treatment and control was the lowest (-4.9 vs -13 and -12.9). These imbalances may have been due to differences in the clinical characteristics of patients across the different centers, as reported by the MAH, including the lower baseline mean cAKUSSI score and patient age in Piešťany compared to Liverpool and Paris centers. Although confidence interval (CI) were large (Piešťany having the largest CI), the point estimate was in favour of the treatment in all sites.

If treatment effect on individual variables of the mAKUSSI is examined, trends towards amelioration in some disease components are observed, such as in osteopenia (T score -0.19 vs -0.05; p=0.045), bone

fractures (8 new bone fractures vs 3, and similar baseline values), and soft tissues ruptures (8 vs 4), although in this last case nitisinone patients with ruptures were lower at baseline compared with control (30.4% vs 24.6% and the number of soft tissue ruptures: 31 vs 27). The positive trend observed in favour of nitisinone on spinal pain could instead likely due to a larger use of analgesic drugs compared to control (almost doubled in the nitisinone group), however, the study protocol did not allow for a thorough investigation about the use of other medications and thus any firm conclusion is difficult to be drawn on this aspect.

Overall, the CHMP view is that it is difficult to infer treatment effect based only on some parameters, and excluding others, as the risk of selection bias is very high. Nevertheless, it is noted that two third of the point estimates of the individual mAKUSSI items favoured nitisinone group. When additional responder analyses, using different definitions of responders, were performed by the MAH, in 6 out of 7 of them, a consistent trend in favour of the treated population compared to the untreated patients was observed. When treatment effect is analysed by patients' u-HGA levels (<300 umol/L and >300 umol/L), it seems that patients with higher u-HGA levels, i.e. those with hypothetical less strict control of the disease, experienced better outcomes. Whereas this subgroup was small (n=14) and included younger patients (difference of about 10 years) who had better baseline conditions (mean mAKUSSI at baseline was 40.8 [21.4 SD] vs 62.4 [28.7 SD] in patients with u-HGA levels <300 umol/L), and thus may have been favoured by earlier initiation of treatment, the CHMP considered that the results of the additional responder analyses together with this finding supported the consistency between treatment effect across disease manifestations and uHGA levels.

Regarding efficacy on other secondary endpoints, the CHMP noted that more patients treated with nitisinone underwent joint replacement. In particular, at month 36, 13 patients (18.8%) treated with nitisinone vs 7 (10.1%) in the control group were submitted to this surgical procedure; the numbers increased to 17 patients (24.6%) vs 10 (14.5%) at month 48, for nitisinone and control, respectively. However, this may be explained by the baseline characteristics of the nitisinone group, which included more older patients compared to control, and also more patients (17, 24.6%) had experienced some joint replacements, compared to the control arm (12, 17.4%). In addition, patients in SONIA 2 were recruited from several countries, and clinical guidelines for performing joint replacements varies between countries, as well as between different clinics within a country. Data about the range of joint motion are conflicting and of difficult interpretation (Figure 15). At 12 and 36 months, some joints show a statistically significant difference that, however, disappears at other time points of the assessment (e.g. at 48 months). Moreover, for some joints (at some assessment times) the point estimate favours the untreated patients. For SF-36 Health Survey, in general a trend is observed in favour to nitisinone (Figure 16) but, the interpretation of these data in a trial not blinded is almost impossible. Of note, at month 48, none of the domains was statistically better in the nitisinone group, even those that were statistically significant at previous time points. The strength of the data does not allow any mention of treatment effect of patient reported outcomes in the SmPC.

2.4.4. Conclusions on the clinical efficacy

In the pivotal study, the efficacy was met for the primary endpoint (uHGA24) and cAKUSSI score (secondary endpoint). Despite the lack of statistical significance in the total population for the mAKUSSI score (secondary endpoint), two third of the point estimates of the individual mAKUSSI items favoured the nitisinone group. Additional efficacy analyses using different responder definitions also demonstrated consistency between treatment effect across disease manifestations and uHGA levels,

The clinical efficacy of nitisinone in the claimed indication is thus considered demonstrated.

2.5. Clinical safety

Introduction

Orfadin is already approved in EU in adults and paediatric patients with HT-1, at the daily dose of 1mg/kg body weight, that is significantly larger than the 10 mg fixed dose proposal for the AKU indication in adults. In the currently approved indication, the drug safety profile is mainly characterised by the risks posed by treatment-induced elevated tyrosine levels that may be associated with toxicity to eyes, skin, and the nervous system; however, Orfadin treatment in HT-1 is commonly associated to eye-related adverse reactions, and less commonly with skin disorders. Blood and lymphatic system disorders were also commonly described during HT-1 treatment in clinical trials, although predominantly mild and moderate in severity.

The clinical safety dataset for the AKU indication is based on 2 clinical studies in patients with AKU.

- The randomized open-label 4-week no-treatment controlled parallel-group dose-response study in patients with AKU (SONIA-1);

- The phase 3 randomized evaluator-blinded no-treatment controlled parallel-group long-term efficacy and safety study in patients with AKU (SONIA-2)

In addition, supportive data from a prospective open-label long-term uncontrolled compassionate-use study in patients with TH1 and data from post-marketing surveillance from the Sobi safety database, corresponding to exposure during a total of 16 557 patient years, were also submitted. These data confirmed the known safety profile of Orfadin in the authorised indication and will not be further detailed in this report.

With the limits of the small sample size (n=8 per group) and short duration of the study (4 weeks) of SONIA-1 study, the adverse events (AEs) reported below is focusing on the long-term efficacy and safety of SONIA-2 study

In SONIA-1 study, there were no SAEs reported and no patient discontinued participation due to an AE. All adverse events were mild, except for one event of moderate back pain in the 4-mg dose group. No notable differences across the treatment groups were observed in hematology, clinical chemistry or vital signs during the study. No patient experienced any corneal effects. Overall, no safety concerns were identified at any of the tested doses of nitisinone over 4 weeks.

Patient exposure

The clinical study program involved a 4-week dose-response study in 40 patients with AKU (SONIA 1), and a 4-year clinical efficacy and safety study in 138 patients with AKU (SONIA 2).

The exposure to nitisinone in the AKU development program is shown in the table 47.

Table 47. Study patient duration of exposure to nitisinone in patients with AKU, by randomised dose (SONIA-1 and SONIA 2 studies)

Duration[a] (Months)[b]	1 mg (N=8)	2 mg (N=8)	4 mg (N=8)	8 mg (N=8)	10 mg (N=69)	Untreated[a] (N=77)
1	8	8	8	8	0	8
> 3	-	-	-	-	69	68
> 12	-	-	-	-	66	67
> 24	-	-	-	-	64	67
> 36	-	-	-	-	59	65
> 48[c]	-	-	-	-	16	18

Source: CSR SONIA 1, Table 10.4 – 1;CSR SONIA 2, Table 10.4.1 – 1.
[a] For patients in the untreated group: Exposed = days in the study.
[b] 3 months = 90 days, 12 months = 365 days, 24 months = 730 days, 36 months = 1095 days, and 48 months = 1460 days
[c] The number of patients treated longer than 48 months. 55 nitisinone-treated and 53 control patients completed the 4-year study (CSR SONIA 2 Table 5).

Adverse events

One hundred and thirty-eight (138) out of the planned total of 140 patients were included in the 4-year efficacy and safety study. Treatment was initiated in all patients randomized to nitisinone. In the untreated group, withdrawn consent was the most common reason for not completing the study, while AE was the most common reason in the nitisinone group. The number of patients reporting at least one AE was comparable for the two treatment groups, however, there were more AE reports in nitisinone-treated than in untreated control patients as shown in the table 47.

Table 47 Overall summary of AEs in 4 year efficacy and safety study (SONIA 2, Safety Analysis set)

SOC PT	Untreated (N=69; PYRs=268)		Nitisinone (N=69; PYRs=260)	
	n (%) [E]	Incidence rate per 10 patient years	n (%) [E]	Incidence rate per 10 patient years
No. of patients with at least one AE	57 (82.6)	2.13	59 (85.5)	2.27
No. of AEs	284		400	
No. of patients with at least one SAE	26 (37.7)	0.97	27 (39.1)	1.04
No. of SAEs	52		57	
No of patients with at least one related AE [a]	NA	NA	18 (26.1)	0.69
No. of related AEs	NA		48	
No. of patients who died	0 (0.0)	0.00	2 (2.9)	0.08
No. of patients with AEs leading to study discontinuation	1 (1.4)	0.04	9 (13.0)	0.35
No. of patients with AEs leading to dose reduction	NA	NA	8 (11.6)	0.31

Source: CSR SONIA 2, Table 10.4.2 – 1, Created using ae.sas, ex.sas, ds.sas and _patientinfo.sas version 1 in S:\Statistical Documents\AKU\Trials\SONIA 2\Final analysis\Final programming\ Data by MG on 12DEC19:10:25:12.
[a] Related to study drug, as judged by the investigator.
Percentage calculated on N (patients in treatment groups)
Abbreviations: AE, adverse event; n, number of patients observed; NA, not applicable; PT, preferred term; PYR, patient years; SOC, body system organ class; SAE, serious adverse event.

The number of patients with AEs was similar between treatment, n=59 (85.5%), and no-treatment groups, n=57 (82.6%). However the number of AEs was higher in the nitisinone group, n=400, vs no-

treatment, n=284. The number of patients with SAEs was similar between the two groups: 39.1% in nitisinone vs 37.7% in untreated.

The following table 48 shows the most common AEs in nitisinone group by Standard Organ Class (SOC) and Preferred Terms (PT).

Table 48. Most common adverse events by SOC and PT (SOC including an AE incidence ≥0.1/10 patient years in nitisinone group), Safety Analysis Set

	Untreated (N=69; PYRs=268)		Nitisinone (N=69; PYRs=260)	
System organ class Preferred term	n (%) [E]	Incidence rate per 10 patient years	n (%) [E]	Incidence rate per 10 patient years
Musculoskeletal and connective tissue disorders	**24 (34.8) [53]**	**0.90**	**31 (44.9) [54]**	**1.19**
Arthritis	6 (8.7) [6]	0.22	6 (8.7) [7]	0.23
Arthralgia	4 (5.8) [5]	0.15	6 (8.7) [11]	0.23
Osteoarthritis	5 (7.2) [7]	0.19	4 (5.8) [5]	0.15
Back pain	2 (2.9) [3]	0.07	3 (4.3) [3]	0.12
Tendon discomfort	1 (1.4) [1]	0.04	3 (4.3) [4]	0.12
Infections and infestations	**11 (15.9) [24]**	**0.41**	**27 (39.1) [56]**	**1.04**
Bronchitis	1 (1.4) [1]	0.04	5 (7.2) [7]	0.19
Urinary tract infection	2 (2.9) [3]	0.07	4 (5.8) [12]	0.15
Pneumonia	0 (0.0) [0]	0.00	4 (5.8) [4]	0.15
Nasopharyngitis	2 (2.9) [2]	0.07	3 (4.3) [3]	0.12
Eye disorders	**8 (11.6) [12]**	**0.30**	**25 (36.2) [65]**	**0.96**
Keratopathy	0 (0.0) [0]	0.00	9 (13.0) [12]	0.35
Eye pain	0 (0.0) [0]	0.00	8 (11.6) [9]	0.31
Dry eye	1 (1.4) [1]	0.04	6 (8.7) [7]	0.23
Lacrimation increased	1 (1.4) [1]	0.04	4 (5.8) [6]	0.15
Ocular hyperaemia	0 (0.0) [0]	0.00	4 (5.8) [5]	0.15
Eye irritation	2 (2.9) [2]	0.07	3 (4.3) [4]	0.12
Investigations	**10 (14.5) [12]**	**0.37**	**24 (34.8) [35]**	**0.92**
Weight increased	4 (5.8) [4]	0.15	14 (20.3) [14]	0.54
Blood alkaline phosphatase increased	0 (0.0) [0]	0.00	3 (4.3) [3]	0.12
Injury, poisoning and procedural complications	**16 (23.2) [29]**	**0.60**	**19 (27.5) [28]**	**0.73**
Fall	6 (8.7) [6]	0.22	8 (11.6) [9]	0.31
Skin and subcutaneous tissue disorders	9 (13.0) [10]	0.34	15 (21.7) [24]	0.58
Pruritus	0 (0.0) [0]	0.00	3 (4.3) [3]	0.12

Gastrointestinal disorders	**13 (18.8) [17]**	**0.49**	**14 (20.3) [27]**	**0.54**
Abdominal pain upper	3 (4.3) [3]	0.11	3 (4.3) [3]	0.12
Toothache	1 (1.4) [1]	0.04	3 (4.3) [4]	0.12
Nervous system disorders	**12 (17.4) [16]**	**0.45**	**14 (20.3) [26]**	**0.54**
Headache	2 (2.9) [2]	0.07	6 (8.7) [11]	0.23
Cardiac disorders	**10 (14.5) [11]**	**0.37**	**9 (13.0) [9]**	**0.35**
Bundle branch block right	6 (8.7) [6]	0.22	4 (5.8) [4]	0.15
Vascular disorders	**12 (17.4) [14]**	**0.45**	**8 (11.6) [10]**	**0.31**
Hypertension	9 (13.0) [9]	0.34	3 (4.3) [3]	0.12
Deep vein thrombosis	1 (1.4) [1]	0.04	3 (4.3) [4]	0.12
Metabolism and nutrition disorders	**13 (18.8) [19]**	**0.49**	**6 (8.7) [7]**	**0.23**
Vitamin D deficiency	5 (7.2) [5]	0.19	3 (4.3) [3]	0.12
Psychiatric disorders	**4 (5.8) [5]**	**0.15**	**6 (8.7) [7]**	**0.23**
Depression	1 (1.4) [1]	0.04	3 (4.3) [4]	0.12
Renal and urinary disorders	**8 (11.6) [16]**	**0.30**	**4 (5.8) [10]**	**0.15**
Renal colic	3 (4.3) [7]	0.11	3 (4.3) [5]	0.12

[E] represents the number of events at each level of summarization.
n: Number of patients observed.
Percentage calculated on N (patients in treatment groups).
Source: Table 10.4.2 - 3

The most commonly TEAEs reported with higher frequency with nitisinone compared to untreated group were within the SOC Musculoskeletal and connective tissue disorder (44.9% vs 34.8% in untreated group; with an incidence rate of 1.11 vs 0.9 per 10 pts year), followed by Infections and infestations (39.1% vs 15.9%, incidence rate 1.04 vs. 0.41 per 10 patient years). The most common localization of infections was the respiratory tract. This difference was seen for all subgroups, i.e., age categories, sites and sexes.

None of the infections was assessed by the investigator or the MAH as related to treatment and most of them were non-serious. None of the infections led to discontinuation of treatment. There was no substantial difference in leucocyte and neutrophil counts between treated and untreated patients and there were no reports of leukopenia or neutropenia. Information on agents for the infections is sparse and no specific agents have been identified as causative for the infections. No connection between nitisinone and infections has been identified in a review of the scientific literature (preclinical and clinical). According to the MAH, there are no indications, either from the study of patients with HT-1 (NTBC study with a total of 291 patients) or from post marketing use (over 15 years), that infectious events are related to nitisinone treatment. In the NTBC study, 8 cases of infections were reported, and none of these was reported in connection with leukopenia and/or neutropenia. Searches of post-marketing cases in the Global safety database (up to February 20, 2019) resulted in 82 ICSRs with 93 events within the SOC "Infections and infestations". This constituted 3.4 % of the AEs cumulatively reported for nitisinone at that time. In patients using nitisinone off-label (AKU), 2 AEs in 70 ICSRs concern infections, both coded as conjunctivitis.

TEAEs of Eye disorders were recorded in 36.2% of patients treated with nitisinone vs 11.6 % of untreated patients, with an incidence rate of 0.96 vs. 0.30 per 10 patient years). In the nitisinone group, 10 patients had tyrosine-related eye disorders, and 9 nitisinone-treated patients developed tyrosine-related keratopathy in one or both eyes as confirmed by slit-lamp examination. One more patient, who could not come for a follow-up visit, was withdrawn from the study due to suspected keratopathy based on symptoms of eye pain. No untreated patients developed keratopathy.

Among the 9 patients diagnosed with keratopathy, 8 patients had other eye symptoms, such as pain, hyperaemia, blurred vision or other signs, before the diagnosis by slit-lamp examination. One patient reported no symptoms before keratopathy was seen by slit lamp at a pre-planned visit. The keratopathy was completely resolved in all 9 patients at a follow-up visit at least 2 months after nitisinone withdrawal. For one patient, there were logistical problems with supplying the 2-mg capsules and this patient was therefore withdrawn from the study. For the remaining 8 patients, nitisinone was restarted at a dose of 2 mg/day after recovery of the keratopathy; 5 of those had recurrent symptoms and were withdrawn from the study while 2 were still asymptomatic at the end of the study, and for 1 patient, keratopathy was discovered at the final visit.

The time to development of the first tyrosine related TEAE ranged from 32 days to about 2 years and 11 months after randomization. The median time to development of eye disorders was 375 days of treatment with 10 mg/day nitisinone.

In the table 49 below, data about vital signs (blood pressure and body weight) are reported.

Table 49. Vital signs (Safety analysis set)_

	Untreated (N = 69)		Nitisinone (N = 69)	
	Value	Change from baseline	Value	Change from baseline
Systolic blood pressure (mmHg)				
Baseline, n	67		69	
Mean (SD)	129.4 (16.5)		126.7 (15.6)	
Month 12, n	63	63	67	67
Mean (SD)	130.2 (14.2)	1.6 (12.7)	131.0 (15.1)	4.4 (15.2)
Month 48, n	53	53	58	58
Mean (SD)	126.4 (13.7)	-3.3 (13.3)	130.7 (13.1)	4.4 (15.2)
Diastolic blood pressure (mmHg)				
Baseline, n	67		69	
Mean (SD)	78.0 (9.7)		76.9 (10.8)	
Month 12, n	63	63	67	67
Mean (SD)	80.0 (9.9)	2.0 (10.1)	80.4 (9.6)	3.6 (9.6)
Month 48, n	53	53	58	58
Mean (SD)	79.6 (9.6)	1.1 (10.3)	80.5 (8.6)	3.8 (11.7)
Pulse rate (bpm)				
Baseline, n	67		69	
Mean (SD)	73.1 (10.7)		72.8 (9.5)	
Month 12, n	63	63	67	67
Mean (SD)	73.2 (14.1)	-0.2 (11.4)	73.4 (10.8)	0.7 (10.6)
Month 48, n	53	53	58	58
Mean (SD)	71.7 (10.2)	-0.5 (8.7)	72.8 (10.0)	-0.3 (12.0)
Body weight (kg)				
Baseline, n	67		69	
Mean (SD)	74.3 (15.8)		74.8 (14.8)	
Month 12, n	63	63	67	67
Mean (SD)	74.3 (15.6)	-0.0 (3.8)	77.7 (15.4)	2.5 (3.6)
Month 48, n	53	53	56	56
Mean (SD)	73.8 (15.1)	0.2 (4.0)	79.0 (15.7)	2.9 (4.8)

n: Number of patients observed
Source: Table 10.4.3 - 6, Table 10.4.3 - 7

The blood pressure (BP) was slightly increased in the nitisinone-treated subjects compared to untreated subjects. In particular systolic blood pressure was increased of about 4.4 mmHg, already after 12 months of treatment. No further increase was evident at 48 months compared to 12 months. Of note, there is no evidence of nitisinone effect on the cardiovascular system in vitro and in vivo studies, all studies showed no nitisinone pharmacologically significant effects on heart rate, blood pressure, and cardiac force.

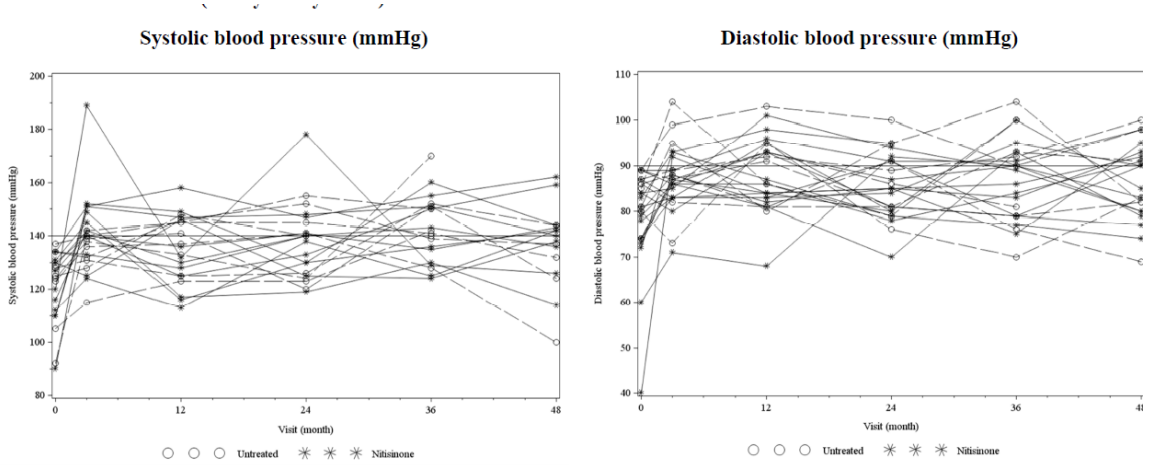
More patients treated with nitisinone in SONIA 2 experienced weight gain compared to non-treated, about 2.9 kg at the end of the 48 months. Most of the weight was gained after the first 12 months of treatment, and after that time point the rate of change seems to be slower.

Additional analyses were submitted to investigate these safety findings at the CHMP request. The percentage of patients with categorical increases in systolic and diastolic blood pressure were presented in the table 50. The individual blood pressures for patients raising to above the reference range at any time, and shift plots of blood pressure were shown in Figures 18 and 19. For the systolic blood pressure, patients are divided into two categories, systolic pressure <140 mmHg or ≥ 140 mmHg, where ≥ 140 mmHg is considered as an elevated blood pressure according to international recognized guideline WHO guideline. For the diastolic blood pressure, patients are divided into two categories, diastolic pressure <90 mmHg or ≥ 90 mmHg, where ≥ 90 mmHg is considered as an elevated blood pressure according to the WHO guideline.

Table 50. Blood pressure, categorised (Safety Analysis Set)

	Baseline		Month 3		Month 12		Month 24		Month 36		Month 48	
	Untreated (N = 69) n (%)	Nitisinone (N = 69) n (%)	Untreated (N = 65) n (%)	Nitisinone (N = 69) n (%)	Untreated (N = 63) n (%)	Nitisinone (N = 67) n (%)	Untreated (N = 61) n (%)	Nitisinone (N = 65) n (%)	Untreated (N = 61) n (%)	Nitisinone (N = 60) n (%)	Untreated (N = 53) n (%)	Nitisinone (N = 58) n (%)
Systolic blood pressure (mmHg)												
<140	51 (73.9)	50 (72.5)	49 (75.4)	50 (72.5)	44 (69.8)	49 (73.1)	45 (73.8)	49 (75.4)	48 (78.7)	49 (81.7)	45 (84.9)	42 (72.4)
≥140	18 (26.1)	19 (27.5)	16 (24.6)	19 (27.5)	19 (30.2)	18 (26.9)	16 (26.2)	16 (24.6)	13 (21.3)	11 (18.3)	8 (15.1)	16 (27.6)
Shift												
Down			9 (13.8)	9 (13.0)	4 (6.3)	6 (9.0)	5 (8.2)	9 (13.8)	10 (16.4)	9 (15.0)	7 (13.2)	8 (13.8)
Unchanged			48 (73.8)	51 (73.9)	52 (82.5)	55 (82.1)	50 (82.0)	49 (75.4)	43 (70.5)	46 (76.7)	44 (83.0)	42 (72.4)
Up			8 (12.3)	9 (13.0)	7 (11.1)	6 (9.0)	6 (9.8)	7 (10.8)	8 (13.1)	5 (8.3)	2 (3.8)	8 (13.8)
Diastolic blood pressure (mmHg)												
<90	62 (89.9)	58 (84.1)	56 (86.2)	61 (88.4)	49 (77.8)	55 (82.1)	56 (91.8)	57 (87.7)	54 (88.5)	49 (81.7)	48 (90.6)	45 (77.6)
≥90	7 (10.1)	11 (15.9)	9 (13.8)	8 (11.6)	14 (22.2)	12 (17.9)	5 (8.2)	8 (12.3)	7 (11.5)	11 (18.3)	5 (9.4)	13 (22.4)
Shift												
Down			4 (6.2)	7 (10.1)	3 (4.8)	5 (7.5)	5 (8.2)	7 (10.8)	4 (6.6)	7 (11.7)	6 (11.3)	6 (10.3)
Unchanged			55 (84.6)	58 (84.1)	49 (77.8)	55 (82.1)	52 (85.2)	53 (81.5)	52 (85.2)	44 (73.3)	42 (79.2)	42 (72.4)
Up			6 (9.2)	4 (5.8)	11 (17.5)	7 (10.4)	4 (6.6)	5 (7.7)	5 (8.2)	9 (15.0)	5 (9.4)	10 (17.2)

Source: VSCAT3_FAS_T.SAS 2020-06-23T07:07:39 Z9FRBE



Source: VS_REF_SAF_F.SAS 2020-06-23T07:08:00 Z9FRBE
Includes patients that were below the reference range at baseline and raise to above the reference range at any time post baseline.

Figure 18. individual blood pressures for patients raising to above the reference range at any time

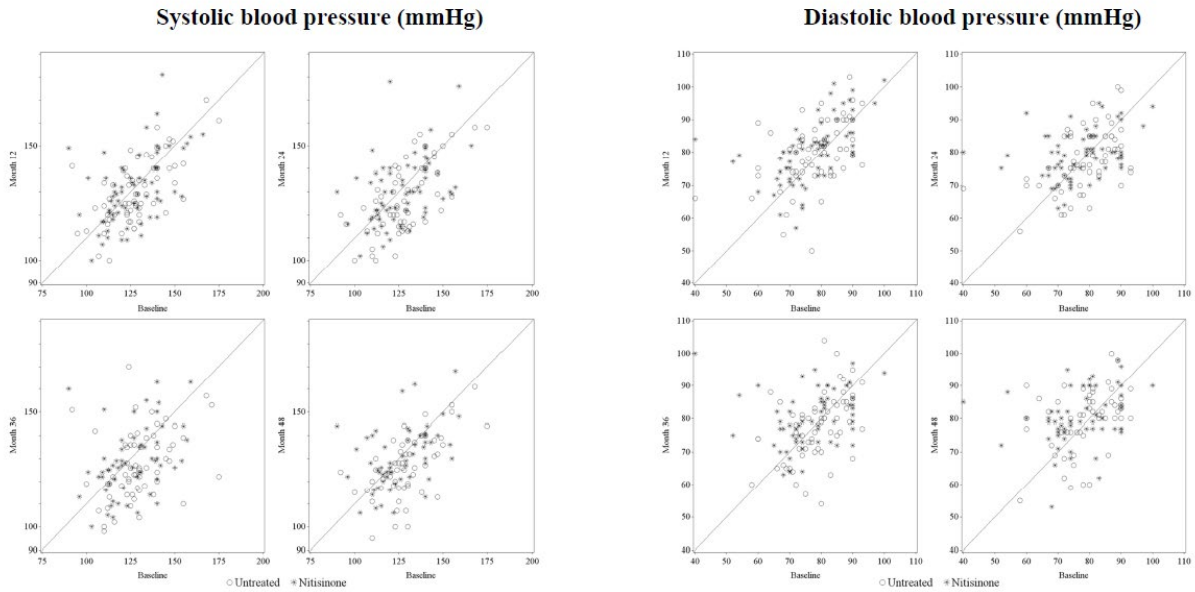


Figure 19. shift plots of blood pressure (Safety Analysis Set)

Whereas, SONIA 2 was not designed to systematically evaluate blood pressure over time (e.g. blood pressure was measured annually at a single time point and the method was not standardized), the following observations can be made based on the presented data:

- the number of patients who had systolic BP >140 mmHg at month 48 was higher in nitisinone (16, 27.6%) than in control (8, 15.1%). However, in the nitisinone group no difference was observed compared to baseline (19, 27.5%). Thus, the observed difference between treated and non-treated patients seems due to a reduction of systolic BP in the control group;
- the number of patients who switched from <140 mmHg to >140 mmHg at month 48 with respect to baseline is equal to patients who switched in the other direction (from >140 mmHg to <140 mmHg), in the nitisinone group. Thus, there was no net change in the number of patients classified in the two BP stages, at month 48;

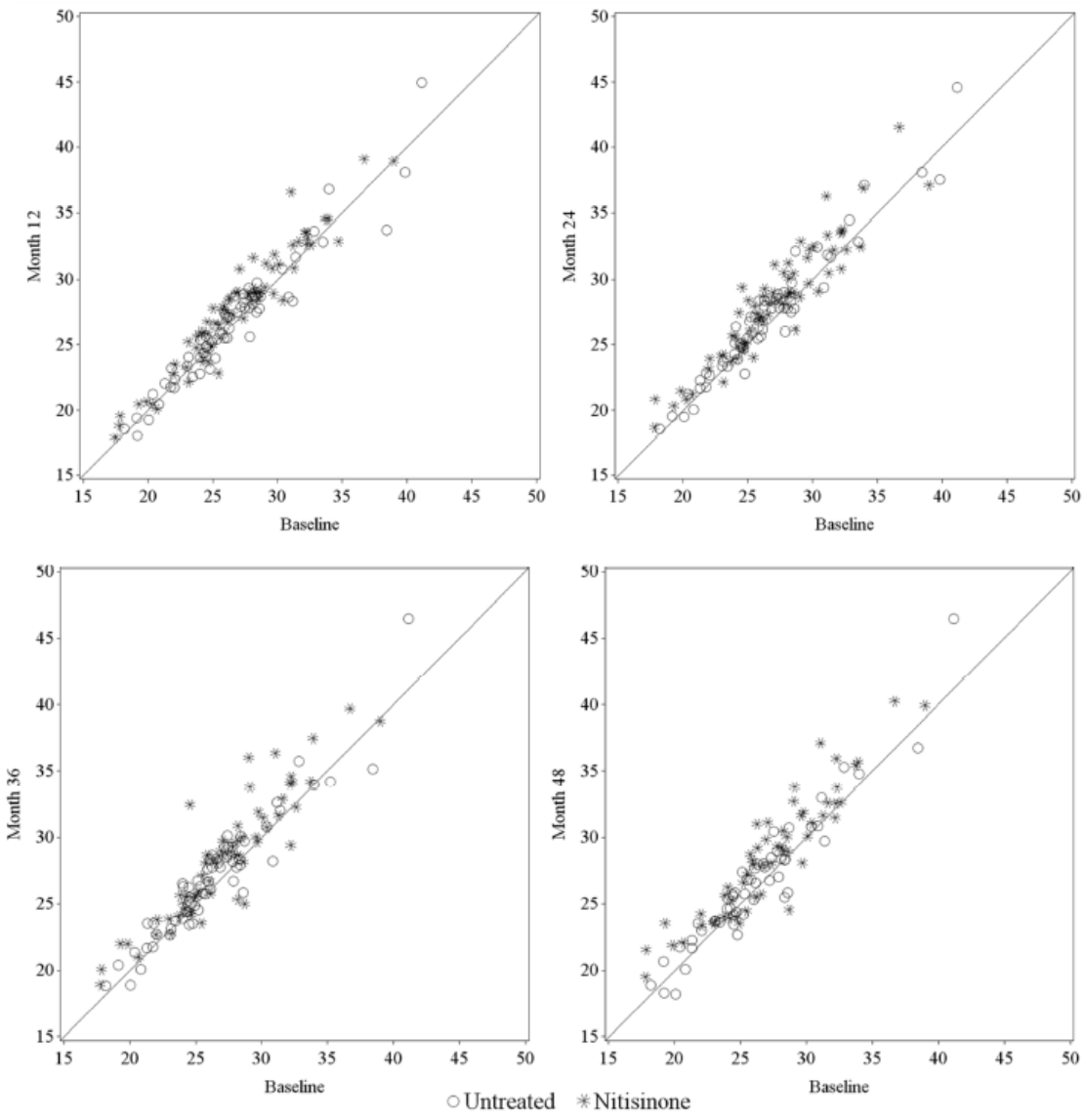
- the number of patients who had diastolic BP >90 mmHg at month 48 was higher in nitisinone group (13, 22.4%) compared to control (5, 9.4%). At baseline the treated patients having >90 mmHg were lower (11, 15.9%) thank at month 48;
- more patients in the nitisinone group shifted from diastolic BP <90 mmHg to >90 mmHg at month 48 (10, 17.2%) compared to control (5, 9.4%) and also compared to those treated patients who shifted from >90 mmHg to <90 mmHg (6, 10.3%); this means that there is a small net increase (n=4, about 7 percentage points) in the number of patients who had >90 mmHg at month 48 compared to baseline.

The distribution of patients by body weight and the shifts among different categories of BMI were shown in Table 51 and Figure 20.

Table 51 BMI, categorised (Safety Analysis Set)

	Baseline		Month 3		Month 12		Month 24		Month 36		Month 48	
	Untreated (N = 69) n (%)	Nitisinone (N = 69) n (%)	Untreated (N = 65) n (%)	Nitisinone (N = 69) n (%)	Untreated (N = 63) n (%)	Nitisinone (N = 67) n (%)	Untreated (N = 61) n (%)	Nitisinone (N = 65) n (%)	Untreated (N = 61) n (%)	Nitisinone (N = 60) n (%)	Untreated (N = 53) n (%)	Nitisinon (N = 58) n (%)
BMI (kg/m^2)												
<25.0	28 (40.6)	22 (31.9)	24 (36.9)	19 (27.5)	24 (38.1)	16 (23.9)	19 (31.1)	16 (24.6)	20 (32.8)	15 (25.0)	20 (37.7)	15 (25.9)
25.0-29.9	30 (43.5)	31 (44.9)	30 (46.2)	31 (44.9)	31 (49.2)	31 (46.3)	32 (52.5)	27 (41.5)	31 (50.8)	25 (41.7)	24 (45.3)	19 (32.8)
≥30	11 (15.9)	16 (23.2)	11 (16.9)	19 (27.5)	8 (12.7)	20 (29.9)	10 (16.4)	22 (33.8)	10 (16.4)	20 (33.3)	9 (17.0)	22 (37.9)
Shift												
Down			1 (1.5)	1 (1.4)	3 (4.8)	2 (3.0)	1 (1.6)	2 (3.1)	2 (3.3)	3 (5.0)	2 (3.8)	2 (3.4)
Unchanged			61 (93.8)	61 (88.4)	57 (90.5)	54 (80.6)	54 (88.5)	50 (76.9)	51 (83.6)	46 (76.7)	45 (84.9)	43 (74.1)
Up			3 (4.6)	7 (10.1)	3 (4.8)	11 (16.4)	6 (9.8)	13 (20.0)	8 (13.1)	11 (18.3)	6 (11.3)	11 (19.0)

Source: VSCAT2_FAS_T.SAS 2020-06-23T07:07:36 Z9FRBE



Source: VS_SHIFT_SAF_F.SAS 2020-06-23T07:07:41 Z9FRBE

Figure 20. Shift plots of BMI

The following observations can be made based on the presented data:

- - Weight gain was observed in 20.3% of patients treated with nitisinone treatment compared to 5.8 % of untreated patients. The increase in BW was already apparent after one year (2.5 kg vs 0 kg in control) and was maintained with a small increase through the whole 4-year period (2.9 kg vs 0.2 kg in control);
- the number of patients who switched from <140 mmHg to >140 mmHg at month 48 with respect to baseline is equal to patients who switched in the other direction (from >140 mmHg to <140 mmHg), in the nitisinone group: so basically, there was no net change in the number of patients classified in the two BP stages, at month 48;
- the number of patients who had diastolic BP >90 mmHg at month 48 was higher in nitisinone group (13, 22.4%) compared to control (5, 9.4%). At baseline the treated patients having >90 mmHg were lower (11, 15.9%) thank at month 48;
- more patients in the nitisinone group shifted from diastolic BP <90 mmHg to >90 mmHg at month 48 (10, 17.2%) compared to control (5, 9.4%) and also compared to those treated patients who shifted from >90 mmHg to <90 mmHg (6, 10.3%); this means that there is a small net increase (n=4, about 7 percentage points) in the number of patients who had >90 mmHg at month 48 compared to baseline.

Serious adverse event/deaths/other significant events

Deaths

In SONIA-2, there were 2 deaths; both occurred in nitisinone-treated patients. One death was due to heart failure, and one was due to myocardial infarction. None of the events was considered, by the investigator, to be related to nitisinone treatment.

The first patient was a, treated with nitisinone 10 mg daily, was hospitalized for an operation of an aortic stenosis (onset before study start). died during the aortic valve replacement, due to an acute myocardial infarction and subsequent cardiac failure. The concomitant illness of aortic stenosis was considered to have accounted for the event. The second patient was a, who was treated with nitisinone 10 mg daily. The patient died at a hospital, but the hospital has not been willing to release medical records. The patient was admitted to hospital due to pneumonia and "infarction" (not specified) causing fluid in the lungs and the patient passed away as a result of complications of the pneumonia 10 days later. This corresponds to Day 1321 in the study, but because of the lack of contact with the patient after Month 36 visit, there is no information on when the patient took final dose of nitisinone. It is assumed, however, that the patient continued with the nitisinone treatment until hospitalization.

SAEs

SAEs are listed in the table 52 below by frequency.

Table 52. Serious adverse events (SAE) by SOC, sorted by incidence rate in the nitisinone group (Safety analysis set)

SOC PT	Untreated (N=69; PYRs=268)		Nitisinone (N=69; PYRs=260)	
	n (%) [E]	Incidence rate per 10 patient years	n (%) [E]	Incidence rate per 10 patient years
Patients with at least one SAE	26 (37.7) [52]	0.97	27 (39.1) [57]	1.04
Musculoskeletal and connective tissue disorders	16 (23.2) [26]	0.60	18 (26.1) [26]	0.69
Vascular disorders	3 (4.3) [4]	0.11	4 (5.8) [5]	0.15
Cardiac disorders	1 (1.4) [1]	0.04	4 (5.8) [4]	0.15
Injury, poisoning and procedural complications	6 (8.7) [7]	0.22	3 (4.3) [3]	0.12
Infections and infestations	1 (1.4) [1]	0.04	3 (4.3) [5]	0.12
Respiratory, thoracic and mediastinal disorders	1 (1.4) [1]	0.04	2 (2.9) [2]	0.08
General disorders and administration site conditions	0 (0.0) [0]	0.00	2 (2.9) [2]	0.08
Renal and urinary disorders	0 (0.0) [0]	0.00	2 (2.9) [2]	0.08
Gastrointestinal disorders	1 (1.4) [1]	0.04	1 (1.4) [3]	0.04
Metabolism and nutrition disorders	1 (1.4) [1]	0.04	1 (1.4) [1]	0.04
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (1.4) [1]	0.04	1 (1.4) [1]	0.04
Product issues	0 (0.0) [0]	0.00	1 (1.4) [1]	0.04
Reproductive system and breast disorders	0 (0.0) [0]	0.00	1 (1.4) [2]	0.04
Hepatobiliary disorders	2 (2.9) [2]	0.07	0 (0.0) [0]	0.00
Nervous system disorders	2 (2.9) [3]	0.07	0 (0.0) [0]	0.00
Congenital, familial and genetic disorders	1 (1.4) [1]	0.04	0 (0.0) [0]	0.00
Endocrine disorders	1 (1.4) [1]	0.04	0 (0.0) [0]	0.00
Eye disorders	1 (1.4) [1]	0.04	0 (0.0) [0]	0.00
Investigations	1 (1.4) [1]	0.04	0 (0.0) [0]	0.00

[E] represents the number of events at each level of summarization
PYRs: Patient years;
n: Number of patients observed
Percentage calculated on N (patients in treatment groups)
Source: Table 10.4.2 - 4

SAEs were reported in a similar proportion of untreated and nitisinone-treated patients, with the highest incidence in the SOC "Musculoskeletal and connective tissue disorders". None of the SAEs was considered by the investigator to be related to nitisinone.

Laboratory findings

Serum tyrosine

The table 53 shows the levels of serum tyrosine in treated and untreated patients.

Table 53. Serum tyrosine concerntrations (µmol/L) at baseline, Month 3 and Month 12 (Full analysis set)

Visit	Statistic	Untreated (N=69)	Nitisinone 10 mg (N=69)
Baseline	n	69	69
	Mean (SD)	64.5 (15.5)	65.3 (14.8)
	Median (min; max)	65.0 (29; 106)	66.0 (32; 104)
Month 3	n	65	69
	Mean (SD)	64.2 (21.1)	950.8 (215.3)
	Median (min; max)	62.0 (26; 130)	939.0 (510; 1464)
Month 12	n	63	66
	Mean (SD)	62.5 (21.0)	919.9 (202.4)
	Median (min; max)	55.0 (23; 119)	925.0 (563; 1530)

Source: Table 10.4.3 - 1

Because of the possibly noncompliant patients, and because there were very few patients on the 2 mg/day dose at any visit, the data in Table 10.4.3 - 1 do not represent a valid comparison of s-Tyr results before and after reducing the dose from 10 to 2 mg/day. Table 58 therefore summarizes all s-Tyr data for the 2- and 10-mg doses at any visit after baseline, for only those 8 patients who decreased the dose to 2 mg/day, and who had quantifiable s-nitisinone when s-Tyr was measured. Decreasing the dose had only a marginal effect on s-Tyr.

In SONIA-2, serum tyrosine concentrations at baseline were comparable in nitisinone-treated and untreated patients (median 66 vs. 65 µmol/L) however after 3 months increased significantly in nitisinone-treated patients (median 939 vs. 62 µmol/L). Tyrosine concentrations in nitisinone-treated patients thereafter remained elevated at approximately the same level until the 48-Month assessment (median 830.5 vs. 61.5 µmol/L). All patients were asked to reduce their protein intake. Patients in the nitisinone group were additionally informed that high tyrosine concentrations may cause eye problems.

In many cases, lowering the nitisinone dose from 10 to 2 mg/day did not result in significantly lower tyrosine concentrations, i.e., concentrations were not reduced to levels under 500 µmol/L (the recommended limit). This finding is in line with the results from SONIA-1 study. In SONIA-1, mean tyrosine concentrations increased with dose of nitisinone. However, all doses, including the 1-mg dose, resulted in daily average concentrations above 500 µmol/L in all individuals, and individual maximum concentrations of approximately 600 µmol/L or more.

Alkaline phosphatase

In SONIA-2, Alkaline phosphatase (ALP) increased in the nitisinone group at all visits after baseline. The greatest change from baseline (median value) was seen at Month 12. After that time, there was no further general increase in alkaline phosphatase. For 4 patients, the changes were considered clinically significant and were reported as AEs; either as increased alkaline phosphatase (3 patients) or as increased liver enzymes. The table below shows the levels of ALP at 12 and 48 months.

Table 54. Alkaline phosphatase (µ/L) at baseline, Month 12 and Month 48 (Safety Analysis Set)

		Untreated (N=69)		Nitisinone (N=69)	
Visit	Statistic	Value	Change from baseline	Value	Change from baseline
Baseline	n	66		69	
	Mean (SD)	74.3 (20.4)		80.8 (25.8)	
	Median (min; max)	73.5 (39;120)		76.0 (43;174)	
Month 12	n	61	61	67	67
	Mean (SD)	71.3 (20.4)	-3.9 (14.2)	93.5 (32.0)	13.4 (15.8)
	Median (min; max)	68.0 (29;115)	-4.0 (-60; 24)	90.0 (48;198)	12.0 (-18;57)
Month 48	n	52	52	58	58
	Mean (SD)	75.9 (24.0)	0.8 (15.7)	89.9 (36.6)	10.0 (21.3)
	Median (min; max)	75.0 (26;132)	0.0 (-35;44)	80.0 (36;246)	9.5 (-55;72)

Source: Table 10.4.3 – 4

Safety in special populations

In the clinical development program for treatment of patients with AKU, all included patients were adults, and 92 to 97 % were White. Subgroup analyses of the incidence of AEs were performed in SONIA-2 with no apparent differences in incidences when analysed by age categories, sites and sexes.

Patients with renal impairment (defined as eGFR <60 mL/min) or with hepatic impairment were not included in the AKU clinical development program.

Safety related to drug-drug interactions and other interactions

No new information on the safety related drug-drug interactions were submitted, which is considered acceptable by the CHMP.

Discontinuation due to adverse events

AEs that led to study discontinuation are presented in table 55 below. Eye disorders were the most commonly reported system organ class and keratopathy the most commonly reported preferred term.

Table 55. Adverse events leading to study discontinuation by SOC and PT (Safety analysis set)

SOC PT	Untreated (N=69; PYRs=268)		Nitisinone (N=69; PYRs=260)	
	n (%)	Incidence rate per 10 patient years	n (%)	Incidence rate per 10 patient years
Patients with at least one AE leading to study discontinuation	1 (1.4)	0.04	9 (13.0)	0.35
Eye disorders	0 (0.0)	0.00	6 (8.7)	0.23
Keratopathy	0 (0.0)	0.00	5 (7.2)	0.19
Eye pain	0 (0.0)	0.00	1 (1.4)	0.04
Cardiac disorders	0 (0.0)	0.00	2 (2.9)	0.08
Cardiac failure	0 (0.0)	0.00	1 (1.4)	0.04
Myocardial infarction	0 (0.0)	0.00	1 (1.4)	0.04
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0 (0.0)	0.00	1 (1.4)	0.04
Endometrial cancer	0 (0.0)	0.00	1 (1.4)	0.04
Pregnancy, puerperium and perinatal conditions	1 (1.4)	0.04	0 (0.0)	0.00
Pregnancy	1 (1.4)	0.04	0 (0.0)	0.00

[E] represents the number of events at each level of summarization
PT: Preferred term; PYRs: Patient years; SOC: System Organ Class
n: Number of patients observed
Percentage calculated on N (patients in treatment groups.)
Source: Table 10.4.2 - 6

Post marketing experience

No information on post-marketing experience with nitisinone in AKU patients was submitted, which is considered acceptable by the CHMP. The long-term safety data available in HT-1 patients confirmed the known safety profile of nitisinone.

2.5.1. Discussion on clinical safety

Orfadin is already approved in EU in adults and paediatric patients with HT-1, at the daily dose of 1mg/kg body weight, that is significantly larger than the 10 mg fixed dose proposed for the AKU indication in adults. In the currently approved indication, the drug safety profile is mainly characterised by the risks posed by treatment-induced elevated tyrosine levels that may be associated with toxicity to eyes, skin, and the nervous system; however, Orfadin treatment in HT-1 is commonly associated to eye-related adverse reactions, and less commonly to skin disorders. Blood and lymphatic system disorders were also commonly described during HT-1 treatment in clinical trials, although predominantly mild and moderate in severity.

The clinical safety dataset for the AKU indication is based on 2 clinical studies in patients with AKU.

- The randomized open-label 4-week no-treatment controlled parallel-group dose-response study in patients with AKU (SONIA 1);

- The phase 3 randomized evaluator-blinded no-treatment controlled parallel-group long-term efficacy and safety study in patients with AKU (SONIA 2)

In addition, supportive data from a prospective open-label long-term uncontrolled study in patients with HT-1 and data from post-marketing surveillance corresponding to exposure during a total of 16 557 patient years, confirmed the known safety profile of Orfadin in the authorised indication.

Maximum exposure of AKU patients to nitisinone 10 mg daily dosage was 48 months. A total of 64 patients were exposed for <24 months, 59 patients were exposed for > 36 months and 55 patients for 48 months and 16 patients for > 48 months.

The most common TEAEs, in the AKU development program, were musculoskeletal and connective disorders, which were expected as they are main manifestations of the underlying AKU disease. However their prevalence was higher in the nitisinone group (44.9%) compared to the control group (34.8%).

The second most frequent TEAEs with nitisinone were infections (39.1% vs 15.9%), in particular in the respiratory tract. The frequency of pneumonia was 5.9% in nitisinone vs 0 in control; the frequency of bronchitis was 7.2% in nitisinone vs 1.4% in control. In the last PSUR, in the HT-1 indication, there were 3 cases of pneumonia observed in an observational study and 2 spontaneous cases; as regards bronchitis, one case was reported in an observational study. Over the 4 annual site visits, leucocyte and neutrophil counts were not notably different in nitisinone-treated compared to untreated patients, and there were no reports of leukopenia or neutropenia. The reason for the observed higher frequency of infections in the AKU indication compared to HT-1 is thus not clear. However, the increase in pneumonia and bronchitis cases was observed in the pivotal study with a randomised control design and the CHMP recommended their inclusion as new adverse drug reactions (ADRs) in the SmPC.

The third most frequent TEAEs were eye disorders, markedly more frequent in the nitisinone group (36.2%) compared to control (11.6%). Eye disorders were also the most frequent cause of treatment discontinuation (n=6, 8.7% of patients in nitisinone group vs no one in the control group). In particular, keratopathy was observed only in nitisinone treated patients (13%). AEs leading to study discontinuation were more frequent in the nitisinone treatment (13%) compared to controls (1.4%). The most frequent AE reported for discontinuation was eye disorders in nitisinone group, which led to discontinuation in 8.7% (n=6) of patients treated with nitisinone and in no one of the patients untreated. Among these, keratopathy was present in 5 patients (7.2%) and 1 patients was affected by eye pain (1.4%).The frequency of eye disorders, including keratopathy, appears higher in AKU compared to HT-1 patients, and the CHMP recommended that this difference in frequency is reflected in the SmPC, ADR Table. Furthermore, levels of tyrosine were markedly increased in AKU patients treated with nitisinone, as expected. After 3 months the levels of tyrosine rose from a baseline value (umol/L) of 65.3 to 950.8. Management of tyrosine elevation is not obvious because dose reduction does not automatically result in significant decreases in tyrosine levels, which are increased also with small doses of 1 mg/day. Moreover, in subjects with keratopathy who reduced the dose to 2 mg/day, recurrence of the keratopathy was observed. Temporary discontinuation of the drug is thus the only choice. A warning has thus been agreed by the CHMP for inclusion in the SmPC, for patients who develop keratopathies included in the SmPC. In patients who develop keratopathies, plasma tyrosine levels should be monitored. A diet restricted in tyrosine and phenylalanine should be implemented to keep the plasma tyrosine level below 500 micromol/l. In addition, nitisinone should be temporarily discontinued and may be reintroduced when the symptoms have been resolved.

Skin disorders are also known to be associated to increased levels of tyrosine. In AKU patients treated with nitisinone skin-related AEs were more frequent in nitisinone group, n=15 (21.7%), compared to untreated, n=9 (13.0%). The frequency of skin disorders in the AKU population has been reflected in the SmPC.

Apparent increases in both systolic and diastolic pressure were observed with nitisinone treatment. Although numerical low, elevations in systolic (4 mm vs -3.3 mm in control) and diastolic values (3.8 mm vs 1.1 mm in control) were already observed after 1 year. However, SONIA 2 was not designed to systematically evaluate blood pressure over time (e.g. blood pressure was measured annually at a single time point and the method was not standardized). Shift plots of systolic and diastolic blood pressure showed no systematic change in blood pressure with nitisinone and in addition no evidence of pharmacologically significant effects of nitisinone on heart rate, blood pressure, cardiac force was generated from non-clinical studies. Thus, there was no clear signal about blood pressure increase associated with nitisinone, however a signal about cardiovascular-related SAEs was identified (see further below). The MAH agreed monitor and provide a cumulative review of the cases related to cardiovascular safety in future PSURs.

Weight gain was observed in 20.3% of patients treated with nitisinone treatment compared to 5.8% of patients in the control. The increase in BW was already apparent after one year (2.5 kg vs 0 kg in control) and was maintained with a small increase through the whole 4 year period (2.9 kg vs 0.2 kg in control). However, it is acknowledged that due to baseline imbalances, the proportion of patients who were overweight and obese at baseline was higher in the nitisinone group compared to the untreated controls. This imbalance could have impacted the results, as those already overweight may be more likely to put on weight than are normal-weight subjects. Moreover, patients treated with nitisinone could have followed the suggested diet, low in proteins, more strictly compared to control group, because aware that high tyrosine concentrations may cause eye problems, during nitisinone treatment. Therefore, no sound conclusion is at present possible on nitisinone effect on body weight.

There were 2 deaths in the nitisinone group and none in the untreated group, during the 4 years of the pivotal study. The deaths were considered not related to nitisinone treatment by the investigator; however, for one of the reported deaths, the patient could have died by pneumonia and/or myocardial infarction.

The total frequency of SAEs was slightly higher in the nitisinone group compared to control (39.1 vs 37.7, respectively). The most common SAEs were musculoskeletal (26% vs 23.2% in control). Of note a larger proportion of SAE of cardiac disorders (5.8% vs 1.4% in control) and infections (4.4% vs 1.3% in control) was observed with nitisinone treatment.

Patients with renal impairment or with hepatic impairment were not included in the AKU clinical development program. However, post-marketing experience in these populations are available in patients with HT-1 and no specific dose adjustment is required for patients with renal or hepatic impairment, as currently recommended in the SmPC information.

Since a few cases of reversible thrombocytopenia and leucopenia were observed in study of patients with HT-1, existing recommendation to monitor regularly platelet and WBC counts is also applicable to AKU patients.

2.5.2. Conclusions on clinical safety

The safety profile of nitisinone in adult patients with AKU is considered sufficiently characterised, and overall qualitative similar to that already known for the HT-1 indication. However, the frequency of several AEs, in particularly those related to tyrosine elevation appears increased (keratopathy, eye

pain), and moreover some new AEs were reported, including infections, particularly in the respiratory system (bronchitis, pneumonia). These differences in frequency are reflected in the SmPC.

In addition, the MAH should submit the following safety data with the next PSUR:

- a cumulative review of the cases related to cardiovascular safety, discussing the cases separately per indication

2.5.3. PSUR cycle

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.6. Risk management plan

The MAH submitted an updated RMP version with this application (Version 5.3): Update of RMP per current template (Rev 2.0 accompanying GVP Module V Rev. 2); addition of new indication alkaptonuria in relevant sections; update of post-marketing data, including data from completed post-authorization safety study Sobi.NTBC-005, in hereditary tyrosinemia type 1; removal of "Hypertyrosinemia-related eye disorders", "Increased tyrosine levels", and "Leukopenia/granulocytopenia" as important identified risks.

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 5.3 is acceptable.

The CHMP endorsed the Risk Management Plan version 5.3 with the following content:

Safety concerns

Summary of safety concerns

Important identified risks	None
Important potential risks	Lack of efficacy
	Developmental and cognitive disorders
	Embryo-fetal toxicity
	Exposure to nitisinone during breast-feeding
Missing information	Interactions with substances known to induce or inhibit CYP3A4
	Use in elderly
	Use in pregnant women

Considering the data in the safety specification, the safety concerns listed above are appropriate; However, the important potential risk "Developmental and cognitive disorders" should be renamed as

"Developmental and cognitive disorders (for the indication hereditary tyrosinemia type 1)", since it is specific for the HT-1 indication.

Pharmacovigilance plan

Ongoing and planned additional pharmacovigilance activities

Study status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 – Imposed mandatory additional pharmacovigilance activities which are condition of the marketing authorization - None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are specific obligations in the context of a conditional marketing authorization or a marketing authorization under exceptional circumstances - None				
Category 3 – Required additional pharmacovigilance activities - None				

Risk minimisation measures

Summary of pharmacovigilance activities and risk minimization measures

Safety concern	**Risk minimization measures**	**Pharmacovigilance activities**
Lack of efficacy	Routine risk minimization measures: SmPC section 4.4	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - Monitored as TME Additional pharmacovigilance activities: - None
Developmental and cognitive disorders	No risk minimization measures identified.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - Specific follow-up questionnaire - Monitored as TME Additional pharmacovigilance activities: - None

Embryo-fetal toxicity	Routine risk minimization measures: SmPC section 4.6	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - Specific follow-up questionnaire Additional pharmacovigilance activities: - None
Exposure to nitisinone during breast-feeding	Routine risk minimization measures: SmPC section 4.3 and 4.6	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - Specific follow-up questionnaire Additional pharmacovigilance activities: - None
Interactions with substances known to induce or inhibit CYP3A4	Routine risk minimization measures: SmPC section 4.5	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - None Additional pharmacovigilance activities: - None
Use in elderly	No risk minimization measures identified.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - None Additional pharmacovigilance activities: - None
Use in pregnant women	Routine risk minimization measures: SmPC section 4.6	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: - Specific follow-up questionnaire

		Additional pharmacovigilance activities: - None

2.7. Update of the Product information

As a consequence of this new indication, sections 4.1, 4.2,4.4,4.6,4.8, 5.1 and 10 of the SmPC have been updated. Particularly, a new warning with regard to patients who develop keratopathies has been added to the product information.The Package Leaflet has been updated accordingly.

2.7.1. User consultation

No justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the MAH. However, the changes to the package leaflet are minimal and do not require user consultation with target patient groups.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Alkaptonuria (AKU) is rare genetic disorder caused by deficiency of homogentisate 1,2-dioxygenase, the enzyme that converts homogentisic acid (HGA) to maleylacetoacetic acid along the tyrosine degradation pathway. Of 626 patients identified worldwide, there are 358 patients in Europe, 208 of whom are found in Slovakia.

The disorder is characterized by excretion of HGA at high levels in the urine, the precipitation of a bluish-black pigment (derived from HGA) in connective tissues, a process called ochronosis, and arthritis of the spine and large joints. HGA undergoes oxidation also in urine determining dark discolouration of urine. The ochronosis occurs usually after 30 years of age. According to one study 50% of patients require at least one joint replacement by age 55 years [Phornphutkul et al 2002]. Other manifestations can include pigment deposition in clear and ear, aortic or mitral valve calcifications or regurgitation and occasionally aortic dilatation, renal stones, and prostate stones.

3.1.2. Available therapies and unmet medical need

Currently, no medicinal products are approved for AKU and nitisinone is sometimes used off-label to treat patients with AKU.

3.1.3. Main clinical studies

SONIA-2 was an international, multicentre, randomized, open-label, evaluator-blinded, parallel-group study with an untreated control group (1:1). The study duration was 48 months with a 12-month interim analysis. Main inclusion criteria were confirmed diagnosis of AKU based on elevated urinary homogentisic acid excretion (uHGA levels); age ≥25 years; any clinical manifestations of AKU, such as clinical ochronosis or chronic back/joint pain.

The primary objective was to demonstrate that nitisinone is superior compared to no treatment in reducing u-HGA24 in patients with AKU after 12 months.

The primary endpoint was urine levels of HGA in 24h after 12 months of treatment with nitisinone. The use of a primary endpoint based on a pharmacodynamic variable is in line with the CHMP recommendation during scientific advice. The CHMP also recommended that, although only HGA is the cause of the ochronosis process that in turn results in the co-morbidities effects of alkaptonuria, there is no historical clinical data to support the assumption that the control of HGA levels in patients with AKU will stop ochronosis.

The main secondary endpoint was the evaluation of treatment effect on the All Alkaptonuria Severity Score Index (AKUSSI score) that measures disease severity in clinical, joint and spine domains. Two versions of the AKUSSI scoring system were used in the study, cAKUSSI which includes eye pigmentation, and mAKUSSI without eye pigmentation in line with the CHMP scientific advice. Indeed, the CHMP recommended to provide data on treatment effect on clinical outcomes to support the claim indication and was of the view that a link should be shown from HGA through ochronosis to clinical outcome.

3.2. Favourable effects

In SONIA-2, Nitisinone reduced u-HGA24 by 99.7% compared to no treatment at month 12 (primary endpoint). At month 12 u-HGA24h (umol/L) were 85.7 (95% CI: 71.8; 102.2) in nitisinone group, vs 26027.9 (95% CI: 21649.6; 31291.8) in the untreated group. The excretion of u-HGA24 reached a nadir at month 3.

The maintenance of this effect was observed over the whole duration of the study, ie 4 years of treatment. At month 48 u-HGA24h (umol/L) were 158.1 (95% CI: 117; 213.6) in nitisinone group, vs 29936.1 (95% CI: 22090.6; 40568) in the untreated group.

In SONIA-2, cAKUSSI score was statistically different between the nitisinone and untreated groups at month 48: change from baseline was 7.4 (95% CI: 2.1; 12.8) in nitisinone group vs 16.1 (95% CI: 10.7; 21.4) in untreated subjects. Adjusted mean (difference nitisinone-untreated) was -8.6 (95% CI: -16.0; -1.2; p=0.023). The cAKUSSI component for eye pigmentation was statistically different between nitisinone and control: change from baseline was 0.5 (95% CI: -0.5; 1.6) in nitisinone group vs 3.0 (95% CI: 1.9; 4.1) in untreated subjects. Adjusted mean (difference nitisinone-untreated) was -2.5 (95% CI: -3.9; -1.0; p=0.001). The mAKUSSI score showed trends towards amelioration in some of its components, such as in osteopenia (T score -0.19 vs -0.05; p=0.045), number of spinal regions with pain (adjusted mean difference nitisinone-untreated: -0.5; 95% CI: -0.9; 0.0, p=0.048), bone fractures (8 new bone fractures vs 3, and similar baseline values; p=0.056), and soft tissues ruptures (8 vs 4). Overall, about two third of the point estimates of the individual mAKUSSI items favoured nitisinone.

3.3. Uncertainties and limitations about favourable effects

The proportion of subjects who had u-HGA levels below the pre-defined cut-off of 300 umol was 88.6% at month 12 but decreased to 59.4% (mean u-HGA24: 158.1) at month 48. This could be an effect due to patient compliance to treatment. The clinical relevance of this finding is unclear as the threshold was set arbitrarily, and measurement of physiological levels of u-HGA24 were very low, even undetectable in some cases; such low levels were not reached by treatment with nitisinone.

No adjustment for multiplicity was performed, thus all secondary outcomes are considered exploratory in nature. The AKUSSI scoring system is currently the only available sensitive tool to assess, in a comprehensive manner, treatment effect on the complex clinical manifestations of the disease. However, it has some limitations, e.g the same score is assigned to morbid variables that may require longer times to develop, and are expression of different stages of disease severity.

Results on cAKUSSI were driven by treatment effect on eye pigmentation, and once the pigmentation variable was removed, as it is in the mAKUSSI, the difference between nitisinone and control was reduced to only -3.6 points and no statistical significance difference was observed (95% CI: -9.6, 2.4; p=0.234). Higher mAKUSSI scores at baseline were observed in patients aged >55 years vs $\leq$55 years (mean score of 69.8 vs 48.2, respectively), in the nitisinone group compared to control. When treatment effect was analysed by age and sex all subgroups reached or exceeded the pre-defined difference of 4 points between nitisinone and control, in both cAKUSSI and mAKUSSI, except for the mAKUSSI score in patients <55 years, suggesting heterogeneity in the untreated and treated groups may have contributed to the lack of effect in the total population.

The positive trend observed in favour of nitisinone on spinal pain was associated with a larger use of analgesic drugs in nitisinone compared to control (almost doubled in the nitisinone group).

More patients treated with nitisinone underwent joint replacement. In particular, at month 36, 13 patients (18.8%) treated with nitisinone vs 7 (10.1%) untreated patients were submitted to this surgical procedure; the numbers increased to 17 patients (24.6%) vs 10 (14.5%) at month 48, for nitisinone and control, respectively. However, patients in the nitisinone group had more severe baseline conditions that could have increased the risk of joint replacement.

Secondary outcomes related to the range of joint motion are conflicting and of difficult interpretation. At 12 and 36 months, some joints showed a statistically significant difference that, however, disappears at other time points of the assessment (e.g. at 48 months). Moreover, for some joints (at some assessment times) the point estimate favours the untreated patients.

Patient reported outcomes (SF-36 Health Survey) showed a trend in favour to nitisinone but, the strength of evidence is very limited since the trial was not blinded. At month 48, none of the domains was statistically better in the nitisinone group, even those that were statistically significant at previous time points.

3.4. Unfavourable effects

The most common TEAEs, in the AKU development program, were musculoskeletal and connective tissue disorders (44.9% vs 34.8% in control), followed by infections (39.1% vs 15.9%), in particular in the respiratory tract. The frequency of pneumonia was 5.9% in nitisinone vs 0 in the control group; the frequency of bronchitis was 7.2% in nitisinone vs 1.4 % in the control group. SAE of infections were observed in 4.4% of nitisinone treated patients vs 1.3% in the control group.

The third most frequent TEAEs were eye disorders, markedly more frequent in the nitisinone group (36.2%) compared to control (11.6%). Eye disorders were also the most frequent cause of treatment discontinuation (8.7% of patients vs 0 in the control group). In particular, keratopathy was observed only in nitisinone treated patients (13%), and in the 7.2 % of cases led to treatment discontinuation. Furthermore, levels of tyrosine were markedly increased in AKU patients treated with nitisinone, as expected. After 3 months the levels of tyrosine rose from a baseline value (umol/L) of 65.3 to 950.8.

Skin disorders were also more frequently reported with nitisinone compared to untreated group, 21.7% vs 13.0%, respectively.

The total frequency of SAEs was slightly higher in the nitisinone group compared to control (39.1 vs 37.7, respectively). The most common SAEs were musculoskeletal (26% vs 23.2% in control). SAEs of cardiac disorders (5.8% vs 1.4% in control) were also observed more frequently with nitisinone treatment.

Dose reduction did not automatically result in significant decreases in elevated tyrosine levels, which instead are increased also with small doses of 1 mg/day; whereas dose reduction leads to relevant increases in u-HGA 24. Moreover, in subjects with keratopathy who reduced the dose to 2 mg/day, recurrence of the keratopathy was observed.

3.5. Uncertainties and limitations about unfavourable effects

Musculoskeletal and connective tissue disorders are main manifestations of the underlying AKU disease. Thus, their higher prevalence in the nitisinone group (44.9% vs 34.8% control) may reflect the limited efficacy of treatment to control disease symptoms.

Over the 4 annual site visits, leucocyte and neutrophil counts were not notably different in nitisinone-treated compared to untreated patients, and there were no reports of leukopenia or neutropenia. The reason for the observed higher frequency of infections, particularly pneumonia and bronchitis, in the AKU population compared to HT-1 population is at present unclear.

There were 2 deaths in the nitisinone group and none in the untreated group, during the 4 years of the pivotal study. The deaths were considered not related to nitisinone treatment by the investigator; however, for one of the reported death, the patient could have died by pneumonia and/or myocardial infarction.

3.6. Effects Table

Table 1. Effects Table for Orfadin for treatment of adults with AKU

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
u-HGA-24h	Urine HGA in 24h at month 12	umol /L	85.7	26027.9	Adjusted geometric mean (ratio nitisinone/untreated): 0.003 (95% CI: 0.003; 0.004); p<0.001	SONIA-2
cAKU SSI	Clinical scoring system including pigmentation		7.4	16.1	Adjusted mean (difference nitisinone-untreated): -8.6 (95% CI: -16.0; -1.2); p=0.023	SONIA-2

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
mAKUSSI	Clinical scoring system excluding pigmentation		8.8	12.4	Adjusted mean (difference nitisinone-untreated): -3.6 (95% CI: -9.6; 2.4); p=0.234	SONIA-2
Unfavourable Effects						
	Musculoskeletal and connective tissue disorder	N (%)	31 (44.9%)	24 (34.8%)		SONIA-2
	Infections and infestations	N (%)	27 (39.1%)	11 (15.9%)		SONIA-2
	Eye disorders	N (%)	25 (36.2%)	8 (1.6%)		SONIA-2

3.7. Benefit-risk assessment and discussion

The ability of nitisinone to largely reduce u-HGA24 in AKU patients is demonstrated. The maintenance of this effect was observed over the whole duration of the study, ie 4 years of treatment. Whilst some observations could question the treatment effect, e.g physiological levels of u-HGA24 were very low and undetectable in many subjects in the pivotal study, these levels were not reached in AKU patients treated with nitisinone group; about 32% of patients who met the u-HGA24 targeted level at month 32 did not maintain it, after 4 years of treatment; the clinical relevance of these findings remain uncertain, especially since the u-HGA24 targeted level was set arbitrarily.

Regarding clinical outcomes, although a statistically positive effect was observed on the secondary endpoint cAKUSSI score, results were driven by the eye pigmentation component, and the statistical significance was lost when this variable was excluded from the score, as reported in the mAKUSSI score. The lack of a statistically significant gain over untreated patients using mAKUSSI did not allow to extrapolate that the reduction in u-HGA24, and the reduced HGA precipitation in the eye may be considered a proxy for clinical benefit. However, the vast majority of the point estimates of the individual items (two third) included in the mAKUSSI score favoured nitisinone over untreated group. In particular, trend towards amelioration in osteopenia, and bone fractures are reported with nitisinone treatment. In 6 out of the 7 additional efficacy analyses using different definitions of responders and combining results on uHGA levels, mAKUSSI score, and eye pigmentation, a consistent trend in favour of the treated population compared to the untreated patients was observed.

While these analyses are supportive of the correlation/consistency between effect on the primary endpoint and clinical outcomes, follow up time of 4 years could have been too short to detect changes in other disease manifestations to result in a statistically significant effect on mAKUSSI total score. Heterogeneity in treatment groups may also have contributed to the lack of statistical significance in the treatment effect on mAKUSSI total score in the total population. Moreover, from the baseline characteristics emerge that the nitisinone group was enriched with patients with more severe disease in some AKUSSI items.

The safety profile of nitisinone in adult patients with AKU is considered sufficiently characterised, and overall similar to that already known for the HT-1 population. However, the frequency of several AEs, in particularly those related to tyrosine elevation appears increased, and moreover some new AEs were observed, including infections, particularly pneumonia and bronchitis. Serious cardiac disorders were

few but occurred 4 fold more frequently with nitisinone treatment compared to control and thus need to be closely monitored post-approval, for the AKU population, in the future PSURs.

3.7.1. Importance of favourable and unfavourable effects

3.7.2. Balance of benefits and risks

Despite the pivotal study met its primary efficacy endpoint, the efficacy on long-term clinical outcomes is at present not completely elucidated. However, based on the observed consistency of treatment effect across disease manifestations and levels of uHGA in SONIA-2, the correlation between treatment effect on the primary endpoint and clinical benefit is demonstrated for Orfadin in the AKU population. The safety profile of Orfadin in the AKU population is in general similar to that already known in the HT-1 population, a few differences in frequency and new ADRs have been observed in the AKU population and are reported in the SmPC.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable

3.8. Conclusions

The overall B/R of Orfadin is positive.

4. Recommendations

Outcome

Based on the review of the submitted data, the CHMP considers the following variation acceptable and therefore recommends, the variation to the terms of the Marketing Authorisation, concerning the following change:

Variation accepted		**Type**	**Annexes affected**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	Type II	I and IIIB

Extension of indication to include treatment of adult patients with alkaptonuria (AKU) for Orfadin; as a consequence, sections 4.1, 4.2, 4.4, 4.6, 4.8, 5.1 and 10 of the SmPC are updated. The Package Leaflet is updated in accordance. Version 5.2 of the RMP has also been submitted accordingly and includes an update in accordance with GVP Module V Revision 2.

Amendments to the marketing authorisation

In view of the data submitted with the variation, amendments to Annex(es) I and IIIB and to the Risk

Management Plan are recommended.

Additional data exclusivity

Furthermore, the CHMP reviewed the data submitted by the MAH, taking into account the provisions of Article 10(5) of Directive 2001/83/EC, and considers that the clinical studies carried out in relation to the new indication were significant (see appendix 1).